Nasal polyps

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NASAL POLYPS

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INTRODUCTION

Nasal polyps are an easily recognisable clinical entity. They are the prolapsed lining of the ethmoid sinuses which block the nose to a variable degree depending on their size. Besides blockage, other nasal symptoms, running, sneezing, post nasal drip, and facial pain may be present as well. On examination polyps usually appear as pale bags which arise in the middle meatus and are relatively insensitive due to a poor nerve supply; they may be palpated by a blunt instrument without local anaesthetic thus differentiated from the turbinates. They are commonly bilateral and, when unilateral, require histological examination to exclude a malignancy. The nasal disease may be limited to the ethmoid sinuses alone, but frequently it extends into the maxillary sinuses or, more rarely into the sphenoid and frontal sinuses. The extent of these changes may be seen on sinus radiographs. The mucosal changes may not be limited to the nose since asthma is present in adults in about 30% of cases (Moloney 1977). This would suggest a more extensive respiratory disease. In children nasal polyps are uncommon and when found before 10 years old are pathonomonic of cystic fibrosis (CF). Polyps do not occur before 2 years since the ethmoids are undeveloped. If a polypoid mass is found then the floor of the anterior cranial fossa should be examined radiographically to exclude a bony deficit.

Treatment is usually by surgical removal although recent studies have suggested intranasal corticosteroids both before surgery (Charlton et al,1986) and following surgery (Pedersen, Prytz, Sorensen,1975;

Mygind 1985) may either cause regression or prevent recurrence.

History

The development of the understanding and management of the condition has been reviewed by Vancil (1969). The Hindus were the first to recognise this condition and, as early as 1000 BC were curetting them. Hippocrates (BC 460-370) recognised them as well and devised a method using a piece of sponge tied to a length of thread which had been passed through the nose in the oropharynx. The sponge was tied to the end in the mouth and then, after positioning in the post nasal space, was pulled through the nose avulsing polyps. The word polyps is derived from the Greek and was first used by Hippocrates. Poly-pos podos which means many footed and described well the processes which ramify in the ethmoid sinuses. It was subsequently used in the Latin, polypus, and authors still incorrectly use the plural, polypi, for the nasal disease.

During the sixteenth and seventeenth centuries snares and forceps similar to those used today were introduced, though the procedure was painful until cocaine anaesthesia was used in the latter part of the nineteenth century.

All nasal polypoid conditions were grouped together until the clinical and histological classification in the nineteenth century helped to differentiate the neoplasms from benign, simple or mucus nasal polyps, which today are referred to as nasal polyps. Billroth (1855), who described their histological classification in the nineteenth century, still considered them neoplastic, whereas by the end of the century Zuckurkandl (1892) understood that they were an essentially inflammatory process. He also demonstrated that the histological

changes in the sinuses were identical to those found in polyps. Because the conditions originated in the ethmoids an infection or inflammation was supposed to occur within the sinuses. Bone is capable of three states, reabsorption, new growth and staying the same. There is no histological evidence for any major bony changes and the features would seem to be confined to the mucosa. These developments have been well reviewed by Berdal (1954).

Recurrence

Nasal polyps are a chronic condition which, following surgical removal, are prone to recurrence. The frequency is varied and one patient may have only one polypectomy during his life; another requires yearly removal. It has been the practice of some surgeons to advocate extensive sinus surgery in patients with severe recurrence (Hughes,1973) but no trials have been performed to test its efficacy or whether extensive surgery alters the disease pattern in those with recurrence. There has also been no study to correlate either the symptoms or past medical history with recurrence, to see if this may help in predicting those who will have recurrent disease.

Histology

Tissue removed from the sinuses, nasal polyps and the bronchial tube (from patients dying from status asthmaticus) are similar. Nasal polyps have respiratory epithelium of ciliated columnar cells and goblet cells, which is supported by a basement membrane that is thickened to a variable degree. The sub-mucosal tissue is grossly oedematous and contains few blood vessels which are mainly capillaries and occasional

nerve fibres. The cellular infiltrate is mainly plasma cells, small lymphocytes, macrophages and, the most striking feature, an eosinophilia. The eosinophilia may be very variable not only from patient to patient but polyp to polyp and part of polyp to part of polyp in the same patient. Eosinophilis are found in 90% of polyps, the remaining are neutrophil polyps. These changes are well described by Freidman and Osbourne (1982). This has been used to classify polyps in the past. Eosinophilic polyps were allergic whereas neutrophilic polyps were infective.

Infection

The cause of the inflammatory process was then, as it is now, uncertain. Pus is rarely found on antral lavage. The American literature, particularly in the 1930s and 1940s, refers to two types of maxillary sinusitis - purulent and hyperplastic. The former is encountered as a true infective sinusitis, the latter is often associated with mucus hypersecretion in which organisms may be cultured sometimes. This has given rise to confusion about the role of infection in the development of nasal polyps. Mucus or mucopus may be washed out of the maxillary sinus in one third of cases. Bacteria are only grown from half of these (17%) and the commonest organism encountered is a non-capsulated haemophilus influenzae (Majumdar and Bull,1982). This organism is a common commensal in the upper respiratory tract and since pus cells are infrequently seen in microscopy it is difficult to implicate haemophilus as a pathogen and in addition the condition does not resolve with appropriate antimicrobodial chemotherapy. This study will be repeated here with fifty two patients.

Asthma

The association of nasal polyps and asthma has long been accepted and has recently been reviewed (Moloney and Collins, 1977). Approximately 20-40% of patients with polyps have co-existent asthma. The figure differs from series to series and it generally starts in adulthood and so is intrinsic or late onset asthma in type. The frequency varies because asthma is a descriptive term for which there is no single accepted definition. It is the chest symptom of reversible wheezy breathing attacks for which no other medical cause, eg heart failure, can account. The aetiology of these attacks is varied. In children it may be produced by an IgE mediated response to pollens and house dust where it is termed extrinsic or allergic asthma. The mechanisms involved in older patients are more obscure and it is difficult to implicate any cause or 'allergen'; commonly patients have normal levels of serum allergen specific IgE, and have negative skin tests. The causes of asthma are probably mutifactorial but many patients have bronchial hyperreactivity to mast cell products and mast cell reactions have been implicated in the tissue reactions (Anon Lancet 1982).

Rhinitis

Nasal symptoms are common, everyone has experienced acute viral rhinitis (common cold) which may proceed to bacterial maxillary sinusitis. Other types of rhinitis are also frequently encountered. Hay fever or seasonal allergic rhinitis is the commonest non infective nasal disease and is due to pollen allergy. Perennial allergic rhinitis

is caused by house dust mite allergy most frequently although feathers, animal danders and moulds all give rise to symptoms. Inhaled allergies may be difficult to diagnose and if one or more skin tests are positive then the condition is labelled an allergic disease. Overall 15% of the population suffer from allergic rhinitis at some time in their life, with hay fever accounting for 10% of these cases (Mygind,1978).

If the skin tests are negative then the condition is labelled vasomotor rhinitis or non allergic rhinitis. The term vasomotor implies a neurovascular reflex and yet this may not be the cause of the symptoms. Patients with polyps may have a long period of symptoms prior to the development of nasal polyps. Bickmore (1981) and Settipane and Chaffee (1977) have suggested that polyps are not allergic despite the commonly held view to the opposite. This thesis will explore mast cell reactions and will give further evidence that a cellular reaction can be found in patients with a non allergic disease.

Allergy

Because of the tissue eosinophilia in 90% of patients with nasal polyps and the association with asthma, allergy has been advocated and in some instances very strongly (Berdal,1954; Blumstein,1966). Unfortunately allergy is an imprecise term which is used in a variety of ways and has changed from its original use by von Pirquet (1906) to describe the altered host reactivity to an antigen - any immune response. It is now used more commonly to mean hypersensitivity. The immune reactions involved cause tissue damage and may be caused by a known antigen reacting with the antigen specific IgE which is attached to the surface of the mast cell. Two molecules of allergen specific IgE

have to sit on adjacent receptors and combine with an allergen. This reaction, which is calcium dependent, causes rapid degranulation of the mast cell and the release of preformed elements, histamine, heparin and other vasoactive and chemotactic factors and the generation of arachidonic acid metabolites, the protaglandins and the leukotrines, the latter are what need to be called slow reacting substances of anaphylaxis SRS-A (Mygind 1978). Serotonin is not found in the mast cells of man but is in dogs, in rats and in some other animals (Mygind 1986).

Although IgE is the main immunoglobulin to produce allergic reactions, another immunoglobulin IgG_4 of the IgG class is also able to produce mast cell degranulation. It differs from IgE by being denatured by heating serum to 56°C for 1 hr, is eluted in the IgG fraction and readily fixes to mast cells. It blocks IgE reactions. It is called the short term sensitising antibody and has been implicated in childhood asthma occasionally (Gwynn, Morrison Smith, Leon Leon, Stanworth, 1978).

There are other causes of mast cell degranulation including other immune responses which result in complexes, notably IgG and IgA reactions. This may be independent of complement activation. The complement cascade itself may have a specific trigger and be activated through the classical pathway or be activated non-specifically through the alternative pathway. Both pathways converge at C_3. Short living fragments C_{3a} and C_{5a} both degranulate mast cells. Mast cells may be degranulated by trauma, drugs and non-specifically. Thus, mast cell degranulation may present in a similar way to a specific known mechanism such as hay fever in the nose. The term allergy may be used clinically, therefore, for a condition for which no allergen can be demonstrated.

Clinically allergy may exist but it is easy to confuse similar nasal symptoms such as attacks of running, sneezing and blockage without an obvious trigger and, because the patient may have one or more positive skin test, label them 'allergic'.

Atopy

The value of skin tests is open to question since the reactions are those occurring at a site distant from the symptoms. The presence of multiple positive skin tests is described as atopy since it is a reflection of a constitutional tendency to develop immediate hypersensitivity reactions.

Allergy as a subject is not well defined nor is the understanding of the mechanisms involved in hypersensitivity tissue reactions. In this thesis allergy will be used for conditions which are produced by a known allergen reacting with an allergen specific IgE or IgG_4 which is on the surface of the mast cell and causes its degranulation. In the nose, hay fever and perennial allergic rhinitis are true allergic diseases where the cause is known eg pollens, animal danders and house dust. This study will review whether this type of mechanism is involved in the production of nasal polyps, and will explore the possible role of mixed grass pollens and house dust mite in the pathogenesis which are the commonest causes of allergic rhinitis.

Mast Cells

Mast cells were described in 1879 by Ehrlich who characterised them by their metachromatically staining granules with basic dyes. The term 'masten' means stuffed and is applied to geese in Germany which were

force fed to produce livers for pate. It described the appearance of the cells with their large number of granules within the cytoplasm.

Cells may be characterised by their morphology, their biochemical reactions and their actions in the organism. Microscopical fixation may change the morphology. It is not possible to classify a cell purely through its morphology nor its fixation characteristics, there has been a tendency to classify mast cells soley through their light fixation characteristics.

Fixation characteristics

Formalin fixation may not fix all mast cells. Hardy and Westbrook (1895) demonstrated that further cells were fixed by fixatives which were alcohol based: Carnoys fixative contains chloroform, ethanol and acetic acid. Smaller mast cells may be identified by this technique. This work has recently been repeated by Enerback and in rats gastro intestinal tract mast cells are classified by fixation into mucosal and connective tissue types. Mucosal mast cells (MMC) are small, found near and in the epithelium and have a smaller number of granules. Connective tissue mast cells (CTMC) are the opposite, they are larger, found deeper often next to blood vessels and have large numbers of granules (Enerback 1981). There has been one study which suggested that fixation may show two different populations in the human gut (Strobel, Miller, Ferguson 1981).

Direct comparison between rat and man is debatable since humans possess a third group of cells, the circulating basophils which rats do not. Miller (1980) has also suggested that mast cells may vary not only from species to species but also organ to organ as well.

The light microscopic findings may not correlate with the ultrastructural details seen on electron microscopy. Mast cells contain electron dense granules which contain scrolls or crystalline patterns which make them easy to identify in the lungs (Caulfield, Lewis, Hein, Austin 1980). Mast cell ultrastructure will be considered in more detail later.

Recent work

Nasal polyp oedema fluid is easy to extract by mincing and centrifuging polyps (Berdal 1954; Donovan, Johansson, Bernich, Soothill,1970). Berdal injected polyp fluid subcutaneously and repeated skin tests. Those patients who had positive skin tests tended to have greater reactions when tested again at the site of injection. He concluded that some polyps were reaginic (or allergic). Donovan showed that the level of IgE was raised in the polyp fluid, but this was irrespective of the results of skin tests, and the history of asthma or hay fever. He thought that polyps were therefore allergic, although John and Merritt (1979) failed to demonstrate that this was an allergy specific to house dust mite.

Electron microscopic examination of polyps by Cauna, Hindover, Manzethi, Swanson (1972) confirmed the histological picture and they commented in passing that mast cells were degranulated, but Cauna stated that the relative lack of nervous tissue was the important feature since autonomic control was lost. Busuttil, More and McGeveney repeated this study (1976) and thought that the polyp mast cells were the important feature but again made no comment on the ultrastructure nor the mechanisms which triggered degranulation.

Whole polyp tissue histamine has been measured and found to be above the normal level in nasal mucosa and that, by histofluorescence the histamine was located in the mast cells (Bumstead et al,1979). Histamine levels of polyp fluid have not been previously measured but tissue removed from children has been challenged with anti human IgE and mixed grass pollen extract. Patients with hay fever released histamine when challenged with allergen extract and those without hay fever also released histamine after the tissue had been sensitised passively with serum taken from a highly atopic subject (Kaliner et al,1973). This study has several limitations; hay fever is not common in polyp patients (Bickmore 1981), nasal polyps are uncommon in children, and this study was conducted in children with cystic fibrosis.

IgE plasma cells have been reported to be more common in polyp tissue stained by immunofluroescent techniques (Whiteside, Rabins, Zettenberg, Crisp,1975). However, the majority of plasma cells are of the IgA type (Bass, Potter, Barney,1974).

These studies do not give a clear indication of the pathogenesis of nasal polyps but suggest that mast cell degranulation may be important but that the mechanism of degranulation has not been unravelled.

Hypothesis

Nasal polyps result from a submucosal reaction which in the majority of cases is mediated by mast cell reactions. The resulting vasoactive products cause a tissue oedema. Because local homeostasis is overcome the oedema may persist where the blood supply is less well developed. The blood supply is least well developed in the paranasal sinuses (Dawes, Prichard 1953). The ethmoid complex is formed by a large number

of small sinuses. The anatomical development, the poor vascularity and the position of these sinuses, allows the lining to prolapse outwards and downward into the nose. Similar changes may be encountered in the other sinuses and in the nasal cavity but to a lesser degree. They may be demonstrated radiographically. Mast cell abnormality should be encountered within the nasal cavity.

The process is dynamic and polyps may change in size and occasionally disappear altogether even without treatment.

As allergy is the commonest cause of mast cell degranulation encountered in clinical practice it may also be the cause of nasal polyps.

The aims

The purpose of this study is to see if mast cell reactions may be involved in the pathogenesis of nasal polyps and to explore whether an allergic reaction may cause their degranulation. The study is divided into four sections. Part one deals with the clinical material to evaluate the incidence of allergic disease and asthma in patients with polyps. The recurrence pattern is also studied to see whether an atopic subgroup can be demonstrated. A study on children with CF will be reported but is limited since it was retrospective and some data were unavailable. The extent of the sinus disease will be evaluated radiographically together with the results of the maxillary sinus washouts to show the incidence of sinus infective sinusitis.

Part two looks at cell morphology. Fixation characteristics of polyp and inferior turbinate will be studied to see if there is any difference in distribution of cells fixed in formalin and Carnoys

fixative. Cell surface IgE will be examined with particular reference to mast cell distribution. The ultrastructural features of mast cells in the inferior turbinate of the normal nose, of inferior turbinate in cases with nasal polyps and of the polyps themselves will be evaluated to see the extent of degranulation.

Part three will look at the oedema fluid to calculate the levels of free histamine, free IgE and other immunoglobulins, allergen specific IgE to HDM and MGP and of IgG_4 both total and allergen specific to HDM and MGP. These results will be compared with the matched serum sample.

Part four studies the release of histamine from polyp tissue and peripheral blood with HDM and MGP extracts and antihuman IgE to see if an allergic reaction may occur. These results will be compared with skin test results.

PART ONE

CLINICAL MATERIAL

PART 1A: CLINICAL PROFILE OF ADULTS WITH NASAL POLYPS

INTRODUCTION

This section covers the clinical case histories of two hundred consecutive patients admitted for polypectomy. The aims of this section are, firstly, to see whether allergic diseases - hay fever, infantile asthma, eczema and penicillin sensitivity - are any more common than might be expected in the general population and, secondly, to see if there is any evidence that those patients, irrespective of the incidence of allergic diseases, form a sub-group within the disease which might predispose to recurrence, and thus implicate indirectly an IgE mediated response as the cause of polyps in some cases.

MATERIALS AND METHODS

Two hundred consecutive patients who were admitted for polypectomy over a 2 year period were studied. Polypectomy was not performed in outpatients but in theatre on sedated patients and generally under local anaesthesia.

On admission patients were questioned on their nasal and past medical history. The results of skin tests, together with the history, were entered onto a standard questionnaire. Basic data included the age at the last birthday, sex, the date of first polypectomy and the number of previous polypectomies. Because of the variability of symptoms and the lengths of history of polyposis, the date of first polypectomy was chosen as the start of the disease since it was easier to confirm from medical records if necessary.

Symptoms: The following symptoms were considered in detail:

Rhinorrhoea (run or running in tables), profuse clear secretions from the anterior nares occurring at least once a week; sneezing, uncontrolled bouts of sneezing again occurring at least weekly. Both these symptoms were usually easy to classify. Partial loss of sense of smell was generally continuous or more rarely variable; this is found as ^{o} smell in the tables. Pain without radiation and occurring around the face, particularly over the bridge of the nose and over the sinuses, was considered as due to the nasal pathology if it was present without visual disturbances, gastrointestinal upset or unilateral eye and nasal symptoms. Itching and running of the eyes occurring also at least weekly and not due to any other cause, was considered as being attributable to the underlying disease. Post nasal drip (PND) was present if the patient complained of the sensation of mucus present at the back of the throat and dripping into the nasopharynx.

Hay fever. Because patients with polyps tend to get attacks of sneezing and running, they often attribute this to hay fever. Careful questioning will reveal that in the majority of cases these symptoms are perennial. Patients with marked seasonal variation in symptoms were easily classified and only those with positive skin tests to mixed grass pollens were considered as positive. While this may exclude the very occasional patient with hay fever and negative skin tests, in view of the polysymptomatology this was felt to be justified.

Eczema. None of the patients had flexural eczema at the time of questioning since in all cases it had occurred during childhood. It was impossible to determine the incidence of infantile eczema.

Drug hypersensitivities fell naturally into three groups: patients with aspirin hypersensitivity, penicillin hypersensitivity and reactions to other drugs - three cases only and two of these were reactions to sulphonamides.

Asthma. Asthma was or had been present if patients had periodic wheezing attacks which required conventional medication for control or reversal. Patients also had to have no evidence of any other chest disease and, since very few of the patients smoked, no symptoms due to smoking. Patients who had an unproductive cough and occasional wheezing were not considered as having asthma. If possible, a normal chest X-ray had to have been performed during the duration of symptoms. A normal radiograph of the chest was required if symptoms were still present. Further details were taken if asthma was still present, and included the severity of the asthma (whether they required oral steroids for control) and details of drug therapy. The relationship of asthma to the development of polyps was studied as was the effect of polypectomy on the course of asthma (see Results).

Previous nasal surgery. Nasal surgery prior to this admission was considered and included any operation before or during the presence of polyps. These were classified as bilateral antral washouts (BAWO), submucus resections of the nasal septum (SMR), radical antrostomy or

Caldwell-Luc (C-L) - in this group were included the only two patients who had had a formal external ethmoidectomy - and finally trimming or removal of the inferior or middle turbinate (Turbs). Patients were usually aware of the nature of their previous surgery but if there was any doubt, medical records were checked and the operation classified accordingly. The only other ENT procedures to be encountered with any frequency were adenoidectomy and/or tonsillectomy and these were grouped together.

Skin tests. These were performed by the prick method to nine common allergies: house dust (HD), house dust mite (HDM), cladasporium moulds (Clad), aspergillus (Asp), mixed grass pollens, B_2, (MGP), feathers, cat danders, milk and eggs together with a negative control. All allergen extracts were produced by Bencard. Reactions were not quantified but only considered as positive or negative. A reaction was positive if a wheal or flare greater than 3 mm occurred within 15 minutes.

Histological examination. It was not the policy of the department to send bilateral polyps for histology, this was reserved for those with unilateral disease. Patients who did not have simple nasal polyps were not included in the study.

Controls

The incidence of hay fever, eczema, asthma, and drugs allergies and positive skin tests is available in the literature and has been used here to compare with the incidence of these in patients with nasal polyps. There are obvious differences since the relative populations are not age matched, but wherever possible references contain reviews of

the literature. No statistical comparisons are made.

Hay fever:	10%	(Harland and McBride 1969) (Mygind 1978)
Asthma total	5%	(Morris-Owen 1976)
childhood	3%+	
late onset	2%	
Eczema	3%	(Solomons 1977)
Penicillin allergy	3-15%	(Sullivan et al 1981)
Aspirin allergy	0.2-0.9%	(Woodbury and Fingl 1975)
Skin tests: one or more positive	20-25%	(Pepys and Duveen 1951) (Davies 1981)

RESULTS

The basic data are shown graphically in Table 1.1 and as cross-tabulations in Table 1.2. This latter table gives the associations between variables at the $P<0.01$ level. The relationship between any one variable and all others may be seen at a glance. There were no missing values. Particular attention will be drawn to those factors which were associated with recurrence and hay fever (Table 1.3).

The age of onset was between 14 and 81 years with a fairly even distribution between 20 and 70. In 10 year periods, there were:

	Ages in 10 year periods						
	10-19	20-29	30-39	40-49	50-59	60-69	70+
Number of patients	7	36	50	31	43	27	6

Those patients who had no previous surgery had a past history of 0 years whereas the longest history of polyposis was 51 years. The median

length of history for patients with recurrence was 9 years. There was no obvious sex difference in the age of presentation, although, as expected from previous studies, there was an overall male predominance (151:49) which is a ratio of 3:1.

Eighty five patients presented for the first time whereas one hundred and fifteen were admitted for a recurrence (46 patients had one previous polypectomy, 27 had two, 15 had three, 9 had four, 5 had five, 2 had eight and 6 had ten or more polypectomies). On the basis of these results, recurrence was collapsed into the following groups: none, one, two, three or four, five or more previous operations. This was to produce groups of reasonable size to facilitate those parameters which may be associated with recurrence.

Symptoms

Because of the length of history of some patients it was impossible to obtain accurate figures for the duration of symptoms prior to surgery. However, over half had nasal symptoms 2 years prior to the first polypectomy. Table 1.1 shows that all patients presented with some degree of blockage folowed by partial loss of smell in 75%. In all, 65% of patients presented with three or more symptoms. Attacks of running and sneezing in the same patient occurred in 40% (both in table). There was a strong association between running or sneezing and eye symptoms, post nasal drip and facial pain (Table 1.2). Those with a history of eczema and aspirin hypersensitivity also tended to have itching and running of the eyes ($P < 0.05$). There was no evidence that any nasal symptoms were associated with a history of hay fever, eczema, drug hypersensitivites nor the results of skin tests.

Past medical history

The results showed that for most of these parameters, hay fever, childhood asthma and penicillin allergies that the rates for patients were similar to normals.

The results together with the literature controls are summarised below:

Parameters	Polyps	Control
Hayfever	10.5	10
Asthma: Total	29	5
Childhood	3.5	3
Late onset	25.5	2
Eczema	11.5	3
Penicillin allergy	7	3-15
Aspirin allergy	5.5	0.2-0.1
Multiple positive skin tests	25%	20-25

All results are expressed in percentages

Hay fever. Twenty one patients (10.5%) had a history of hay fever and in all but two seasonal variation was no longer present. There was some evidence that penicillin hypersensitivity (7%) occurred more frequently in patients with hay fever and strong evidence that hay fever was associated with positive skin tests. (As expected from the definition this was particularly so for mixed grass pollens ($P < 0.001$)). But also these patients tended to have other skin tests which were positive: house dust, feathers, cat danders, milk, and eggs (all P

<0.001).

Skin tests. Fifty six per cent of patients had completely negative skin tests and 25% of patients had three or more positive skin tests. The allergens to which patients were commonly positive were house dust (48 patients), house dust mite (52 patients), feathers (48 patients) and mixed grass pollens (51 patients). There was a clear tendency for multiple positive skin tests. There was no difference below the age of 60 between the age of the patients and the results of skin tests. It would seem that skin tests were more likely to be negative over 60. The numbers were too small for any useful statistical results (Table 1.4).

Eczema. Twenty three patients had a history of childhood eczema and there was some suggestion that patients later had more sinus surgery and that there was a trend with asthma and aspirin hyper- sensitivity. The grouping of these variables probably reflected the association between asthma and recurrence, sinus surgery and recurrence and eczema and recurrence (Table 1.3, see later in text).

Aspirin hypersensitivity. All but one of the eleven patients also had asthma ($P < 0.001$). There was an increased tendency for these patients also to have more frequent sinus surgery and antral wash-outs ($P < 0.01$) This grouping was also explained in the factors associated with recurrence.

Asthma. Seven of the fifty eight patients with a history of asthma had the disease during childhood alone and two further patients did not

require medication for control of symptoms at the time of study. Although nasal polyps were three times more common in males, thirty eight men had asthma (25%) compared to twenty women (41%), ie a woman with polyps was nearly twice as likely to have asthma as a man with polyps. The distribution of the age of onset of nasal polps was similar for patients with and without asthma (polyps alone, mean age of onset 42 years, SD 16 years, asthmatics, mean age of onset 40 years, SD 16 years). Asthma, on the other hand tended to develop first (mean age of onset 34 years, SD 16 years).The longest interval between the onset of asthma and that of polyps was 47 years; the converse was 16 years. Asthma occurred within 5 years of the onset of nasal polyps in thirty patients. This may suggest a sub-group who were likely to develop both conditions as a result of an underlying condition. There was no effect of surgery on asthma in twenty six cases, and the chest was improved in sixteen patients; only one patient complained that polypectomy made the chest worse and she developed asthma 1 month after her second polypectomy. The severity of asthma may be indicated by the medication; those with severe disease required oral steroids. There were no obvious differences in recurrence rate of polyps in the eight patients who required oral steroids as opposed to the twenty six who required salbutamol inhalers and beclomethesone inhalers and the fifteen who were controlled on inhaled salbutamol alone (Table 1.5).

Previous ENT surgery. Thirty nine patients had other nasal surgery. The majority had more than one procedure, thirty four had sinus wash-outs (BAWO) and eighteen had SMR, eighteen Caldwell-Lucs (C-L) and seven had turbinate surgery (Turbs). The tendency for multiple procedures to be performed may be seen in Table 1.2.

The only other ENT operation to be encountered frequently was adenotonsillectomy in seventy two patients. It is difficult to obtain accurate figures for the numbers of adenotonsillectomies performed over a long period; even though the numbers have decreased over the last 20 years it is doubtful whether more than about 5% of the general population have had this procedure. The figure of 35% for these patients suggests that there could well be an increase in nasal symptoms during childhood prior to the development of polyps.

Factors associated with recurrence of nasal polyps

The natural history suggests that recurrence is common, and one hundred and fifteen of the patients studied had recurrent disease. As previously stated some of the associations of variables found in Table 1.2 are probably related to recurrence of disease. All the variables were cross-tabulated with the patients who had recurrent polyposis. Those variables which were significantly associated with recurrence ($P < 0.05$ or less) are reported here (Table 1.3).

As might be expected, in addition to the variables considered in Table 1.2, the length of the history and the age of onset were important. The only symptom which suggested any relation with recurrence was the loss of sense of smell. This may reflect severe mucosa disease which might be the explanation why nasal surgery both

before and during the disease was significantly more common in patients with recurrence. Eczema, aspirin hypersensitvitiy, asthma and a positive skin test to milk were also associated with recurrence. Although these variables are associated with recurrence they may not have equal weight. Through the technique of multiple regression it was possible to analyse the relationship between the number of polypectomies and the significant variables.

In the one hundred and fifteen patients with recurrence it was possible to account for 46% of the variation in recurrence. The length of history of polyposis accounts for 23% of the variation, major sinus surgery 12% and asthma 7% (age of onset and positive skin tests to milk both 2%). The importance of the length of history was to be expected and the only other contributions come from multiple nasal surgery and asthma. Over half the recurrence variations remained unexplained.

Recurrence and Allergy. Obviously other variables which were not considered may be important. However, it was possible to state from the variables considered that hay fever, penicillin hypersensitivity and multiple skin tests were not associated with recurrence of polyposis and did not seem in this respect to form a sub-group within the heteogenicity of the disease. That is although there was an atopic subgroup the recurrence pattern was no different.

Table 1.1

This table is a descriptive summary of the clinical information. I shows the sex incidence, the numbers of patients with a particul symptom. Both refer to patients who have both running and sneezing. The next section shows the number of patients who have had oth operations. The fourth part looks at the incidence of diseases that a often considered allergic and this is continued in the last secti which looks at the percentage of patients with positive skin tests (BA bilateral antral wash out; SMR submucus resection; Ts and A adenotonsillectomies).

Table 1.1: Descriptive information on 200 polyp patients

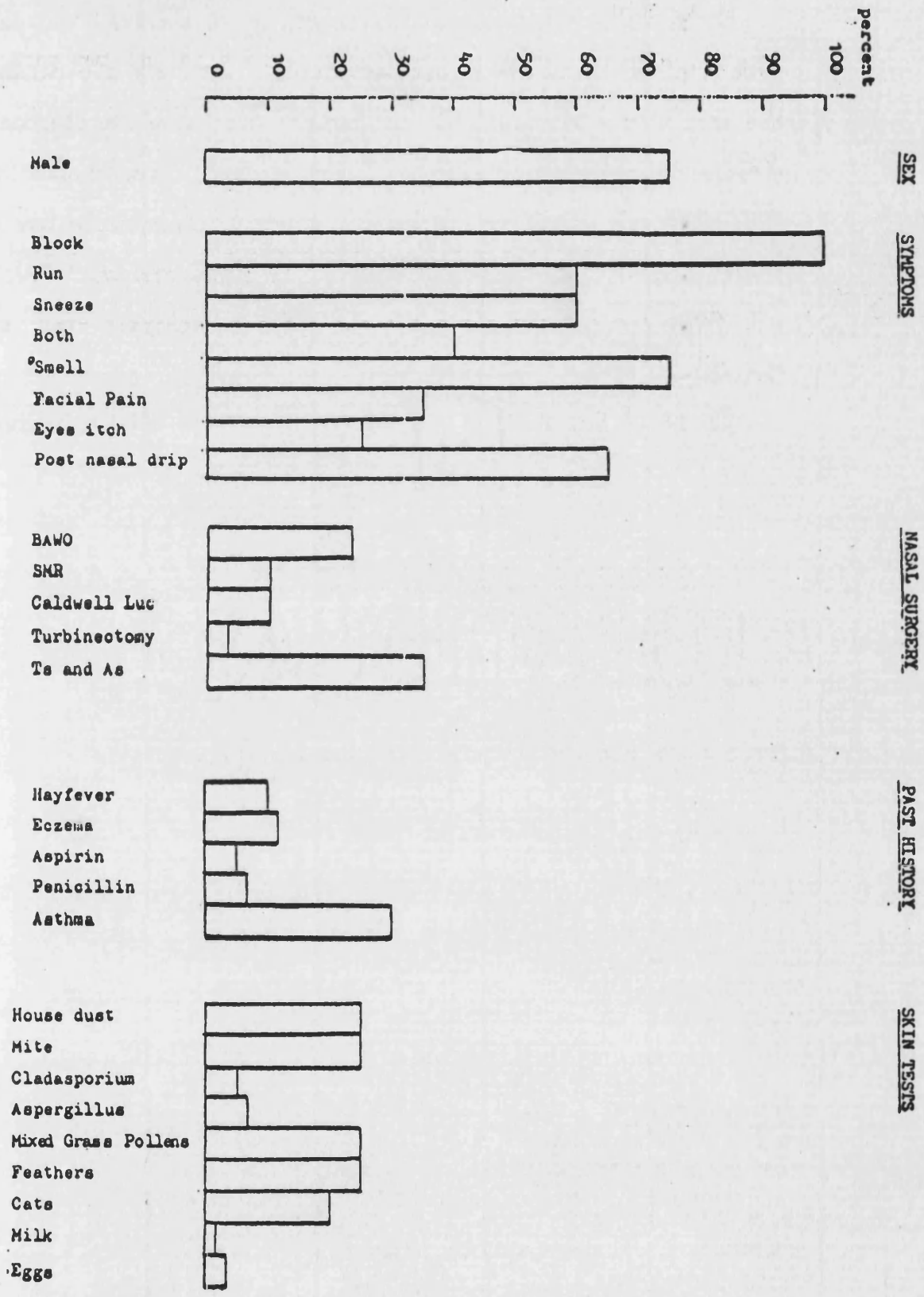


Table 1.2

While Table 1.1 looks at the incidence of symptoms and parameter this table compares the numbers of patients with any two parameters a the statistical association. The numbers are below the diagonal and t degree of association above. Chi squared comparisons are u throughout and since the values are grouped, for example hay fever positive skin tests to house dust, mixed grass pollens (MGP) feathe cat danders, milk and eggs, no further analysis was conside necessary.

Values of $p<0.01$ or ** and $p<0.001$ or *** are used throughout.

Table 1.2: Cross tabulations and associations of variables

	Run	Sneeze	Smell	Pain	Eyes	P.N.D.	Hay Fever	Eczema	Aspirin	Penicillin	Asthma	B.A.W.O.	S.M.R.	C.L.	Turbs	T&A	H.D.	H.D.M.	Clad	Asp	M.G.P.	Feathers	Cat	Milk	Eggs	Male	Length +	Total
Run	82	***			***																							117
Sneeze	95	93			***			**																				117
Smell	39	40	56		**	***																						150
Pain	42	41	40	27																								70
Eyes	80	84	103	59	39																							51
P.N.D.	12	12	16	7	9	12																						130
Hay Fever	12	16	7	9	11	15	5										**											21
Eczema	15	21	14	7	11	15	1	4																				23
Aspirin	7	5	9	7	7	8	4	7	6																			11
Penicillin	6	9	8	6	7	11	5	4	0	3	13																	14
Asthma	34	34	48	20	18	41	10	11	10	4																		58
B.A.W.O.	18	21	26	15	8	22	4	7	6	3	13																	34
S.M.R.	11	11	13	7	3	15	1	2	1	2	4	9																18
C.L.	10	9	16	5	3	10	3	6	2	1	9	11	5															25
Turbs	5	4	5	4	3	6	2	2	3	1	4	4	4	2														18
T&A	43	44	55	34	22	50	6	9	6	5	23	18	7	6	2													72
H.D.	33	27	37	18	13	32	11	3	2	4	17	5	7	2	3	15												49
H.D.M.	35	29	40	25	16	37	8	4	4	5	20	7	8	2	5	20	41											52
Clad	7	5	9	3	4	3	2	0	1	0	3	1	0	1	0	3	8	6										8
Asp	8	7	10	4	3	4	3	0	0	0	3	2	0	1	0	5	8	7	5									10
M.G.P.	30	33	40	18	14	29	21	9	2	8	18	6	3	3	3	12	27	23	7	8								51
Feathers	34	27	37	17	16	29	12	5	3	6	18	4	7	2	4	17	34	34	8	8	25							48
Cat	31	24	32	14	13	25	10	3	2	5	14	5	7	2	4	13	31	31	6	9	22	35						43
Milk	3	1	4	0	1	1	4	0	0	0	1	1	0	1	0	1	3	4	1	1	4	5	5					5
Eggs	4	4	5	1	3	3	5	1	0	0	3	2	0	1	0	3	4	5	1	2	6	5	6	4				7
Male	67	82	110	46	34	92	15	16	9	8	38	25	15	14	7	57	40	43	7	8	37	32	35	4	6			151
Length +																												
Total	117	117	150	70	51	130	21	23	11	14	58	34	18	18	7	72	49	52	8	10	51	48	43	5	7	151		

+ Length of polyp history was coded as, zero, 1-4 years, 5 or more years for the cross tabulations.

Table 1.3

Recurrence is a problem in the management of patients with nas polyps. This table looks at recurrence to see if there are any facto associated with this. Recurrence is more severe in patients with asth and those who have aspirin hypersensitivity and since the patients ha worse disease they tend to have more nasal surgery. It is important t note that recurrence is not associated with hay fever, penicill allergy and positive skin tests.

Chi squared tests are used throughout and * is $p<0.05$ and ** is $p<0.01$

Table 1.3: Factors affecting the recurrence rate of nasal polyps

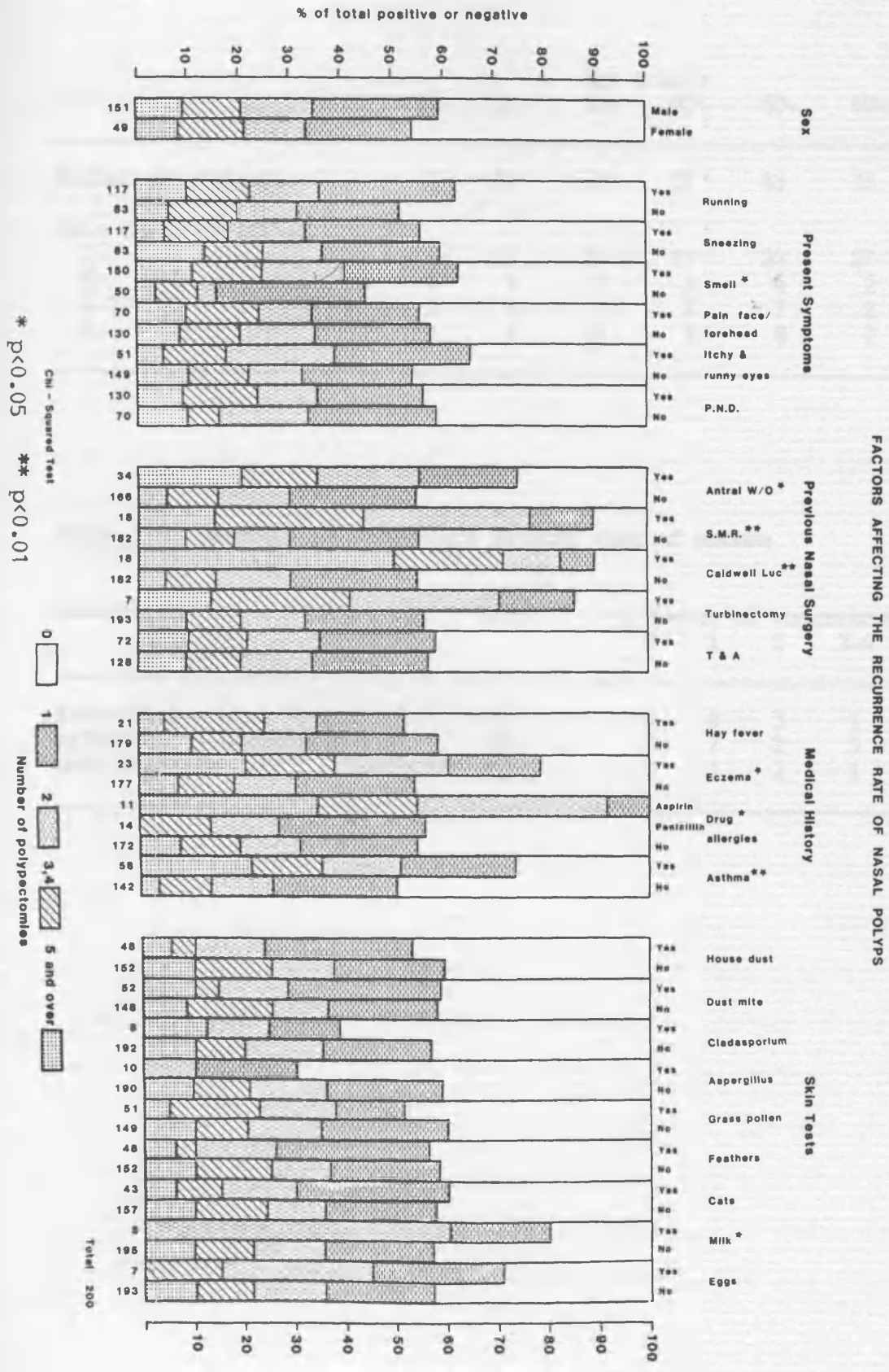


Table 1.4: Skin test results with age

	Age groups					
	19	20+	30+	40+	50+	60+
Number of patients	7	36	50	31	43	33
Skin tests positive:						
0	4	13	25	19	24	27
1	0	9	5	5	4	2
2-3	2	9	5	2	7	2
4+	1	5	15	5	8	2

Table 1.5: Severity of recurrence against that of asthma

Medication	Total	Number of recurrences				
		0	1	2	3-4	5+
Salbutamol	15	4	4	3	1	3
Salbutamol + bectomethazone	26	7	7	2	5	5
Oral steroids	8	2	1	2	1	2

DISCUSSION

The two main theories about the pathogenesis of nasal polyps are the infective and the allergic which may not necessarily be exclusive (Wilson, 1976). It is perhaps unwise to seek a single aetiology on a common clinical and histological picture.

Clinically, hay fever is produced by either tree, or more commonly, grass pollens causing seasonal attacks of sneezing, running and itching of the eyes together with nasal blockage, and wheezing in some cases and is mediated via an IgE response (Ishizaka and Ishizaka, 1967). If hay fever were either to predispose or cause polyps it should occur more frequently in patients with polyps and be related to an earlier onset of polyps. The incidence of hay fever varies from country to country and is between 3% and 20%; it is about 10% in England (Harland and McBride 1969, Mygind 1978). This study shows that patients with polyps have an incidence similar to that of the general population (10.5%). The history of hay fever preceded the onset of polyps generally and in only two cases was it still present with polyps. It would appear that hay fever is a separate unrelated nasal disease which has no bearing on either the development or severity of nasal polyps. Indeed, the analysis suggested is less common recurrence in patients with hay fever.

It is easy to confuse the symptoms of rhinitis, attacks of blockage, sneezing and running, with hay fever, and patients, because they are aware of people who suffer from true hay fever, will say that they too have it. The symptoms of a cold are also similar but a careful history will distinguish those who have perennial symptoms from those who have hay fever either alone or with a persistent rhinitis but are subject to

acute summer exacerbations. These findings confirm the impressions of Bickmore (1981) who stated that hay fever was uncommon in patients with polyps. There does seem a genuinely atopic group in this study who have hay fever, penicillin allergy, and multiple positive skin tests and whose reactions are presumably mediated through an IgE response.

The incidence of penicillin allergy is between 3% and 15% in the general population (Sullivan et al,1981) and, to all intents, this group of patients can be considered as normal; 7% admitted to sensitivity to penicillins. Although the mechanisms involved in the production of penicillin hypersensitivity are variable, an appreciable number may be associated with an IgE response (Ibid).

A positive skin test to grass pollens is found in 25% of the population and this was the figure found in this group (Mygind,1978). The same is true for the skin tests except for reactions to house dust and house dust mite. These are positive in at least 15% of the normal population and here the figure was 26% which is at the upper limit of normal for both allergens suggesting an increased sensitivity to dust mite and other perennial allergens in dust (Davies,1981). Since feathers contain dust mites and household dust is composed mainly of skin and dust mites there is considerable overlap both in allergens and therefore the reactions in patients to these materials. The skin tests show that both MGP and HDM are the most likely allergens to be implicated in allergic reactions and thus form the basis of further work.

It has been suggested that skin tests in patients were more likely to be positive in patients under 40 (Pepys and Duveen,1951). This study was unable to confirm this; only patients over 60 had less reactions.

There appears to be an even distribution of skin test reactions before this. They showed also that the presence of positive skin tests had no effect either on length of history of polyposis nor on the recurrence rate (Ibid).

Asthma. The relationship between asthma and nasal polyps has been long and widely recognised. In general there is a great degree of overlap between diseases affecting the nose and the chest. In childhood patients with cystic fibrosis may present after 2 years of age with nasal polyps (Schwachman, Kukzychi, Mueller, Flake,1962) and over a third of them have nasal symptoms with this disease.

Unfortunately, earlier authors who commented on the relationship between asthma and nasal polyps did so in an anecdotal manner. Francis (1929) stated that polypectomy in atopics caused asthma whereas Samter and Lederer (1958) considered that polypectomy in non-atopics caused asthma. This has led to the frequent assumption that polypectomy causes asthma. It appears that these authors were unware of the difference between a coincidence, the natural incidence, an association and causation. Indeed, many authors today still find difficulty in appreciating these relationships between diseases.

Asthma or wheezy breathing occurs during the life of about 5% of the normal population; the majority of cases occur during childhood and here the mode of action is commonly via an allergen specific IgE on a mast cell (Morris-Owen, 1976). In this study seven cases had childhood asthma (3.5%) and this fits well with the expected norm. The majority were late onset in type (24.5%). This is much more common than expected and confirms the association between these two diseases. Moloney (1977)

noted that the onset of asthma was distributed around the date of first polypectomy and that surgery has probably no effect on the development of asthma. This study confirms those findings. The onset of asthma tended to be earlier than that of polyps and surgery had little effect on the development of asthma or the severity of the disease once established; if anything, the chest was improved. The earlier onset of asthma is to be expected since those patients who have presented with polyps may yet develop asthma. Those who develop asthma before polyps are a complete group whereas those who develop asthma folowing first polpectomy are an incomplete group, they may still develop asthma.

Patients with aspirin hypersensitivity, asthma and nasal polyps are a well recognised sub-group (Delaney,1976), which may occur in up to 8% of patients; it was 5.5% here. The mechanisms both in these patients and those with adult asthma alone are unclear, but in the main they do not fit the classical IgE release pattern. There is some suggestion that the aspirin action is caused by inhibition of prostaglandin synthesis (Sczeklik, Gryglewski and Czerniawska-Mysik,1975) but this does not explain either the asthma or the polyps in the absence of aspirin ingestion.

Recurrence

Recurrence of nasal polyps is one of the problems facing every ENT surgeon in the management of these cases. Although there are advocates for extensive sinus surgery in severe recurrence (Hughes 1973), no real evidence has ever been presented that such an approach works in practice. The pathology does not lie in the structure of the sinuses although the anatomy of the ethmoid facilitates the tissue oedema. The

lining of the sinuses will grow back in the same manner and will undergo the same changes which occur in the submucosal layer. In practice, after a Caldwell-Luc procedure, the polyps issue not only from the ethmoids but prolapse out of the antrostomy in the inferior meatus. Intranasal ethmoidectomy is neither a complete operation nor a safe one. Not only is the surgery blind (the patient may also become so) but it renders the middle turbinate unstable. If this is removed, the main landmark of the nose is lost, making subsequent surgery difficult.

Support for the view that pathology lies within the epithelium is found in this study when the factors associated with recurrence are considered, eg patients with asthma, eczema or aspirin hypersensitivity. These patients undergo more surgery. Not only is polypectomy encountered more frequently but also other surgery on the sinuses and nose both before and during polyposis. Other nasal surgery does not improve the condition; if it did so then surgery before the onset of polyps would lead to infrequent polypectomy.

Medical treatment has been tried to shrink polyps away and to prevent recurrence. The mainstay of this approach are corticosteroids: beclomethesone diproprionate sprays topically or systemic prednisolone. Earlier reports using topical steroid spray suggested that polyps regressed, but these studies also mentioned control of symptoms (Mygind et al,1975; Deuschl and Drettner,1977). This earlier enthusiasm for topical treatment has not been supported by further publications. Oral prednisolone may cause polyps to go in certain patients, but those who are on steroids for control of asthma tend still to have recurrence. There seems little justification in using a drug long-term which has well recognised complications when the disease may be controlled by

surgery which may be performed safely under local anaesthetic. The only study at present published on patients treated with topical beclomethasone diproprionate to prevent recurrence was on small numbers (Karlsson and Rundcrantz,1982). A further study has failed to confirm this benefit and demonstrated a natural incidence of spontaneous regression in some cases (Lang and McNeill,1983).

It is important that further studies are performed on larger numbers and that treatment groups are comparable with respect to variables associated with recurrence. Essentially any study should ensure comparability of the length of polyposis, previous nasal surgery and asthma. It is not necessary to consider present symptoms as these have been shown to be unrelated to recurrence. There is only one control of recurrence and it is the regimen which gives rise to fewer operations.

In conclusion, the clinical profile does not fit in with a disease which is atopic in nature. Hay fever, childhood asthma, penicillin hypersensitivity and multiple positive skin tests did not occur any more frequently in the population at large. Although there was a genuinely atopic sub- group they were not associated with more severe recurrence, so that it would appear that a classic IgE response is unlikely to cause polyps. Asthma is associated with nasal polyps and further study on the mechanisms involved in the production of nasal polyps may well help eventually in understanding those involved in the bronchial mucosal oedema.

PART 1B: BACTERIAL INFECTION OF THE MAXILLARY SINUS IN NASAL POLYPS

INTRODUCTION

Bacterial infection is believed by some authors to be responsible for nasal polyps (Majumdar and Bull 1982). The air spaces in the ethmoid sinuses are obliterated by the oedematous mucosa but there is little evidence histologically for infection in the bones around the ethmoid sinuses (Berdal 1952). Rather than direct infection, it is felt that infected secretions from the maxillary sinus (which opens into the ethmoid sinuses) travel over the ethmoid sinuses and these cause and perpetuate the oedema. Despite the confusion in the usage of the term 'sinusitis' which was covered in the introduction, the diagnosis of maxillary sinusitis is made from the post nasal drip, the radiographic changes in the sinuses and the results of antral wash out during polypectomy. An alternative theory to infection by the bacteria is an allergy to them which is present in the nose and sinuses. In both cases if the bacteria are removed then the condition should resolve.

Mucus hypersecretion is caused by a number of aetiologies and a post nasal drip is not a diagnostic symptom. Radiological changes in the sinuses are not a good indicator of fluid in the maxillary sinuses (Pfleiderer, Drake-Lee and Lowe 1984) and the meaning of the changes will be covered in the discussion. Irrigation of the maxillary sinus will produce a fluid return in some cases only and in half of these no organism is cultured (Majumdar and Bull 1982).

The aim of this section is to look at any evidence for bacterial maxillary sinusitis in patients and to discuss the likelihood of this as

a common cause of nasal polyps.

MATERIALS AND METHODS

Fifty two unselected patients were admitted for nasal polypectomy and bilateral antral wash outs. All had a thorough history and examination, and on admission sinus radiographs were performed. None were on antibiotics.

Radiographs. All patients had routine sinus radiographs taken within 12 hours of surgery. Information was obtained on the frontal, ethmoids, maxillary and sphenoid sinuses and was coded as follows: 0, no evidence of disease within the sinus; 1, mucosal thickening which was less than half the cross sectional area of the sinus; 2, mucosal thickening greater than this; 3, a fluid level; 4, a completely opaque sinus and 5, an absent sinus radiographically and only found in the frontal sinuses.

Antral washouts. Lavage was performed via the inferior meatus according to the standard technique as described by Ritter (1977) during the polypectomy. If no material was found on direct aspiration, 10-15 mls of N saline was instilled directly into the sinus and aspirated back into a syringe. The results of irrigation were coded as follows: 0, no return or a few flakes of mucus; 1, scanty material produced either on direct aspiration or irrigation and 2, large amounts of fluid.

Bacteriological investigation. If material was aspirated directly it was sent without irrigation fluid, otherwise returned material from the

wash out was collected and sent for microscopy and for culture. The material was examined for pus cells and results were classified as having none or few pus cells 0, or large numbers of cells, 1. The remaining material was cultured routinely on agar plates which included standard plates, electrolyte deficient medium, chocolate agar, blood agar and anaerobic blood agar. The organisms were classified as follows 0, no growth; 1, haemophilus influenzae; 2, streptococci and 3, others.

RESULTS

Table 1.6: The extent of radiographic changes in the sinuses
N=52 expressed as a percentage.

		Radiographic changes					
		0	1	2	3	4	5
Frontals	R	27	27	27	2	13	4
	L	29	35	22	0	10	4
Ethmoids	R	12	25	18	0	45	0
	L	16	23	15	0	46	0
Antras	R	11	25	30	5	29	0
	L	9	21	30	11	29	0
Sphenoids		59	25	10	2	4	0

Table 1.7: **The extent of radiographic changes in the maxillary sinus compared with the return from irrigation, pus cells in wash outs and organisms cultured from 104 maxillary antra.**

	Return			Pus cells		Organisms			
Radiographic changes	0	1	2	0	1	0	1	2	3
0	9	2	0	11	0	10	1	0	0
1	9	10	3	17	5	17	1	3	1
2	10	10	12	23	9	24	2	6	0
3	3	0	6	6	3	4	0	0	5
4	2	18	10	22	8	18	7	3	3

Clinical findings. This group of patients was comparable to those studied previously. Twenty-nine patients (56%) presented for the first time, fourteen (27%) had asthma. The sex ratio, incidence of symptoms and previous surgery were similar.

Extent of radiological changes

It is well known that the frontal sinuses vary in development and may be absent and a figure of 4% absence is in keeping with this. All the other sinuses were present. The first table shows the extent of the disease in the sinuses. The radiographic findings may represent both mucosal disease and secretions, mucus which may be very viscid or thin fluid. A fluid level (3) may be present with or without gross mucosal thickening. An opaque sinus may have fluid in it. With these reservations a progression of mucosal disease may be determined 0, 1, 2,3 and 4. There are no statistical differences between the sinuses,

but the sphenoid sinus has least in the way of mucosal disease. It is noteworthy that mucosal disease extends into the frontal sinuses in addition to the maxilla and ethmoids in most cases. As expected the most extensive disease is in the ethmoid sinuses.

Correlations with wash out findings

If the maxillary antra were clear radiographically little or no material was produced on proof puncture. When the sinus had mucosal thickening (1,2,) then nineteen of the fifty-four irrigations produced no return, twenty scanty material and fifteen large amounts of material. Two of the thirty opaque sinuses gave no return (4), Table 1.7. There was a trend for more fluid to be found the greater the degree of radiographic changes. In the nine sinuses which were judged to have a fluid level, six produced large amounts of material on irrigation.

Organisms cultured on the return from antral wash outs

Bacteria were cultured from thirty-one of the one hundred and four wash outs. Pus cells were present in twenty-five wash outs and the combination of both bacteria and pus cells in sixteen wash outs.

Haemophilus influenzae was cultured from eleven wash outs and was found in one antrum without pus cells and no radiographic changes. Streptococci were grown from half the wash outs with fluid levels (haemophilus was not cultured here) and was grown as commonly as haemophillus. Eight other wash outs grew four other organisms, the most frequent was Branhamella Catarrhalis which was found in three cultures.

DISCUSSION

The role of bacteria in the pathogenesis of nasal polyps may either be as an infective or an allergic agent. In either case bacteria should be present and the condition should respond to appropriate therapy to eradicate the organism. Antibiotics have little therapeutic benefit in nasal polyps and their role is not usually covered in medical text books.

Infection is diagnosed by isolating the offending organisms, if possible producing a similar lesion in animals by inoculation and finally curing the condition by appropriate treatment. Nasal polyps are confined to man and have never been produced in animals, this may be due to anatomical differences. Treatment by corticosteroids may resolve the condition in half the cases. Corticosteroids should exacerbate the condition since they suppress immunological reactions which defend the host and thus argue against a prime infective process.

This study sent all wash out returns for culture and examination for two reasons. Firstly to demonstrate the incidence of fluid in the maxillary sinus and secondly to detect any organisms which might be present as commensals.

The sinuses are generally believed to be sterile although there are no data available to support this. The nose on the other hand is easily reached but swabs are usually taken from the anterior nares and post nasal space. Gwaltney and Hayden (1982) reviewed the literature and concluded that up to one half the adult population would grow a commensal organism from the nose. The most commonly encountered bacteria were staphylococci, streptococci and haemophilus influenzae.

Since the sinuses are in direct contact with the nasal cavity organisms would be present in the sinuses in some cases. Haemophilus influenzae was grown from one patient who had no pus cells and no abnormality radiographically, which would support this hypothesis.

When the mucosa is abnormal it may be easier for organisms to grow and still not be pathological. Half the wash outs had no pus cells and yet an orangism was cultured. In only sixteen of one hundred and four wash outs were both organisms and pus cells found which suggests that bacterial infection is unlikely to cause the majority of polyps.

It is possible that infection may arise because the mucosa is diseased rather than cause the disease itself. The role of haemophilus influenzae in chronic bronchitis is such a case. The organism invaded already diseased tissue.

Even authors who strongly support the role of bacterial infection in nasal polyps (Majumdar and Bull 1982) are unable to obtain a return from the maxillary sinus in 40% of cases and when they did obtain fluid found no growth in half the wash outs. Unfortunately this study did not send cultures from all sinuses nor report on the extent of disease radiographically.

Sinus radiographs

The reliability of sinus radiographs to detect infection has been criticised together with their role in diagnosis (Axelson et al 1970; Vourinen Kauppila and Pulkkinene, 1962 and Watt Boolsen and Karle 1977). These studies compared wash outs with irrigation and a further study which included ultrasound (Pfleiderer, Drake-Lee and Lowe 1984) concluded that if the sinus radiographs were normal then there was

little chance of any abnormality on wash outs. They also concluded that except for a fluid level all other changes were not diagnostic of chronic sinusitis. A further study which compared direct inspection of one hundred and seventy antra in one hundred and fifteen patients with detailed radiographs showed that radiologically normal sinuses may have minor mucosal changes (13 of 53 sinuses) and that occasionally an abnormal sinus may have a normal mucosa on inspection (28 out of 117 sinuses). The discrepancy lay in minimal changes diagnosed radiographically (Pfleiderer, Croft and Lloyd 1986). It may be concluded that if the radiographs are abnormal then some degree of mucosal abnormality is likely to be present in the maxillary sinus.

It is possible to extend these conclusions to the other sinuses which are not as accessible for invasive investigation. Although polyps are a disease of the ethmoid sinuses, the process extends into other sinuses in the majority. No other radiographic abnormality was noted including bone erosion. The radiographic changes may be associated with secretions, but the findings are not diagnostic of infection in the maxillary sinuses in the majority of cases.

Since this is so, it is somewhat illogical that surgical treatment is limited to the ethmoids and maxillary sinuses. Extensive surgery to the maxillary sinus while ignoring the frontal sinus would seem ill thought out when extensive changes are seen radiographically.

In conclusion, this section shows that mucosal disease extends into the majority of the sinuses and that bacterial sinusitis is unlikely to cause the majority of polyps. It is possible that bacterial allergy does predispose to the development of polyps but since organisms were not found commonly this is unlikely. Allergic reactions are mediated by

IgE and challenge with antihuman IgE should degranulate mast cells irrespective of the allergen. This mechanism will be studied further in section 4.

PART 1C: CYSTIC FIBROSIS AND NASAL POLYPS IN CHILDREN

INTRODUCTION

Cystic fibrosis is a multisystemic disease which affects the exocrine glands. It is an autosomal recessive disease which occurs in 1 in 2000 live births. The exocrine sweat glands are unable to produce a dilute sweat and values of above 60m Eq of sodium in two consecutive tests are diagnostic. The disease produces gastrointestinal and respiratory symptoms and may present at birth with the failure of the neonate to pass meconium. The gastrointestinal symptoms may produce severe debility with failure to thrive. Conversely some children have little in the way of gastrointestinal symptoms and present with recurrent respiratory tract infections. These may result in severe lung damage which is produced by persistent colonisation with bacteria particularly staphyloccoci and pseudomonas. The respiratory disease may affect the nose and the paransal sinuses.

Nasal symptoms range from minor catarrhal episodes to frank mucopurulent discharge and nasal polyps. The ethmoid sinuses are developed adequately only after 2 years of age to give rise to polyps so any lesion developing before 2 years must be investigated to exclude herniation of the brain and its covering. Polyps may be the presenting sign of cystic fibrosis. It holds that any child with nasal polyps should have cystic fibrosis excluded.

The disease was described in 1936 by Fanconi and his colleagues and was called cystic fibrosis by Andersen in 1938. The association between nasal polyps and cystic fibrosis was first mentioned by Lurie in 1957, although the first documentation of the nasal and sinus changes was

presented by Bodian in 1952. He noted, mainly from post mortem specimens that the nasopharynx, nasal cavity and sinuses were frequently filled with mucopurulent material and that the mucosa was congested. Lurie reported three cases, two of which had nasal polyps. The first major review of the nasal manifestations was by Schwachman and his colleagues in 1962. He reported on the findings of seven hundred and forty two patients and found polyps in fifty cases. Eight patients presented with polyps and the commonest ages to develop polyps was between 5 and 10 years, with only two cases presenting before 3 years. They also suspected that polyps were not associated with atopy.

A similar study was reported 10 years later by Neely and others (1972) who found polyps in fifteen of ninety three cases. They found that sinus radiographs were always opaque, but felt that this was not diagnostic since changes occur in 30% of children anyway (Marsh and Washburn, 1940). They found allergy in nine cases of nasal polyps but this they felt was not significant.

Except for Bodian, all the previous studies originated in the United States. Most British studies were case reports - the largest number reported was by Berman and Coleman (1977). They presented seven cases, six of whom had nasal polyps. The first large study was performed by Drake-Lee and Pitcher Wilmott in 1982 which looked at the differences in presentation between cases with nasal polyps and those without. The findings will be summarised here but this study will look at what evidence there was for allergy and sinus infection in these cases.

MATERIALS AND METHODS

Only nine cases of nasal polyps and cystic fibrosis were under review at the Hospital for Sick Children (London) at the time of study. An additional eleven children had been treated in the past 12 years, two of whom had died and one had moved abroad. Some data were missing from the records. The control group comprised ninety seven patients who were under review at the hospital. A standard questionnaire was used throughout the study and data was transferred onto punch cards prior to analysis.

Statistical analyses. All statistical analyses were performed using multiple analysis of variance (ANOVA) by the multiple regression approach. The statistical package for social sciences which was in use at the University of London Computer Centre was used throughout.

General parameters. These were available on all children and included sex, birth weight, age at presentation, presenting complaint (meconium ileus, diarrhoea, failure to thrive, recurrent upper respiratory tract infections and nasal polyps).

Bowel symptoms. These were coded as follows, 0 - no problems, 1 - occasionaly diarhoea but good weight gain, 2 - frequent diarrhoea and poor weight gain, 3 - severe diarrhoea, abdominal distension and failure to thrive.

Chest symptoms. The most current chest radiograph with anterior posterior and lateral projections was scored using the Crispin and

Norman method (1974). Respiratory function was checked in patients old enough and the peak flow rate, internal capacity of forced expiratory flow at 25% vital capacity by flow volume curves: values were expressed as percentage predicted value for height. Positive colonisation in the lungs was considered as present if three consecutive sputum samples grew either staphyloccus aureus or psudomonas aeruginosa.

Allergy. Variable numbers of these parameters were available from the records (see Results). A history of eczema, hay fever, asthma and positive skin tests was recorded. Asthma can be difficult to diagnose in patients with the respiratory manifestations of the disease. It was considered as present if reversible airways obstruction occurred which responded to standard medication. Skin tests were positive if a wheal occurred which was greater than 2mm diameter. Where possible serum IgE levels were compared between the two groups.

Maxillary sinus washouts. During the period of study nine patients had sinus wash outs and details were available on a further two. Fifteen maxillary sinus wash outs were performed by the standard inferior meatal approach. The sinus was aspirated and then a few mls of warmed normal saline were irrigated. Direct culture of material was made if possible and if not irrigation fluid was set. The material was cultured by the standard methods in the bacteriology laboratory. A sputum sample was taken on admission and the cultures were compared with wash out results.

RESULTS

There were several significant differences and associated trends between children with cystic fibrosis and nasal polyps compared with those without polyps. Two patients presented with polyps and there were more males in the polyp group (16/20, 58/97) which may explain the significantly heavier birth weights (polyps, 3.75 kg, control 3.09 kg, $P < 0.001$). Polyp patients presented later with a mean age of 31.5 months compared with 15.1 month in the control group, $P < 0.01$. This may be due to the relative absence of meconium ileus in patients with polyps (1/20, 25/97).

Bowel habits. Meconium ileus has been shown to occur less frequently and the same lack of of gastrointestinal symptoms continued in the first year before treatment ($P < 0.005$).

Scale	Polyp	Control
0	7	11
1	6	22
2	6	39
3	1	25

Respiratory function. Respiratory function and chest radiograph scores were comparable between the two groups except that vital capacity was better preserved in polyp patients. Pseudomonas colonisation although equal in incidence occurred slightly later in polyp patients and may have given rise to less damage. Polyp patients with a mean difference of 24 months end may have resulted in less lung damage and this explains the better preserved vital capacity. Patients with polyps also had a lower incidence of staphylococcal colonisation (7/18, 67/97, $P < 0.005$).

Allergy. All allergic complaints were comparable between both groups of patients. There were no obvious differences between the incidence of positive skin tests and total IgE levels.

Parameter	Polyp	Control
Eczema	1/16	9/96
Hay fever	2/10	20/96
Asthma	1/9	23/97
Skin test +ve	5/15	53/95

Sinus washouts. Organisms were cultured from five wash outs only, in only one case was the organism the same as that grown in the sputum, psuedomonas. Nine of the corresponding sputa grew organisms. The results of antral lavage and sputum samples are set out below:

Results of cultures of antral wash outs and sputa

Patient	Washout	Antral culture	Sputum culture
1	1	NG	NG
	2	NG	Staph + Haemophilus
2	3	Strep + Haemophilus	Haemophilus + Pseudomonas
3	4	NG	Staph
4	5	NG	Pseudomonas
	6	Pseudomones	Pseudomonas
5	7	Haemophilus	NG
	8	NG	Haemophilus
6	9	NG	NG
7	10	E,Coli, Pneumococcus	Haemophilus
	11	E,Coli	Pseudomonas
8	12	NG	Staph
9	13	NG	NG
10	14	NG	NG
11	15	NG	NG

NG = no growth

DISCUSSION

Adults with nasal polyps are not directly comparable with children who have both cystic fibrosis and nasal polyps. There are some similarities, adults may have coexisting chest disease such as asthma, and children with cystic fibrosis certainly do have lung damage. Children with cystic fibrosis are not directly comparable with other children so that the control group consisted of children with the disease but without polyps. (Some of the control group may go on to develop polyps.)

There was a male predominance which is similar to adults and polyps are associated with the respiratory rather than the gastrointestinal manifestations of the disease. This may answer the question raised by Schwachman et al (1962) when they suggested that there would be an increase in the numbers of children with polyps with improved survival. The improved survival is found in controlling the gastrointestinal symptoms in the first few years of life. The incidence of nasal polyps in Schwachman's study was 8%, the incidence in cases under review was nine in one hundred and six approximately 8.5%.

The respiratory manifestations were similar between both groups as was atopy. It was difficult to document hay fever in the patients reviewed retrospectively but the orders of this and other parameters in cases under review were similar. Although allergy may occur in patients with polyps, both Schwachman et al (1962) and Nealy et al (1972) considered that nasal polyps were not necessarily an allergy manifestation. Nealy et al concluded that 'Nasal polyps did not correlate with allergy although approximately one third of patients with

cystic fibrosis had allergies'. This study confirms this conclusion.

The role of sinusitis is more difficult to determine. Frequent courses of antibiotics given to children with cystic fibrosis may sterilise the sinuses. Ten of the wash outs grew nothing on culture. The material aspirated from the sinuses is quite unlike that taken from adults and children without cystic fibrosis. Children with cystic fibrosis have an inspisated brownish mucus that smells musty. In adults it is either mucus or pus and may smell offensive. It would appear that classical sinusitis did not coexist in children with cystic fibrosis and nasal polyps.

Pseudomonas in particular has been shown to be related to lung damage and it was grown from only one sinus wash out as opposed to four sputum samples. This finding, together with the less frequent culture of organisms does not support the hypothesis that the sinuses serve as a reservoir for chest disease. It would seem unlikely that bacterial sinusitis is associated with the development of nasal polyps in children with cystic fibrosis. In this respect and the lack of allergic diatheses, they would seem comparable to adults with nasal polyps.

PART TWO

MAST CELL MORPHOLOGY

INTRODUCTION

The routine histology of a polyp was presented in the introduction and, briefly, the picture is that of a gross oedomatous submucosa covered by a respiratory epithelium. The most noticeable cell on routine histological staining with haemotoxylin and eosin, is the eosinophil. The majority of polyps contain eosinophils and together with the gross oedema, they suggest inflammation.

This section evaluates the role of mast cells in these reactions. As mentioned earlier mast cells may be a heterogenous group of cells which can be differentiated by fixation characteristics. Mast cells also bind IgE avidly to their surface and reactions involving this immunoglubulin is one method by which they are degranulated.

The histological findings from one method of tissue preparation may not be applicable to those from another, and one set of fixation techniques may change the bioactivity of the tissue. While Carnoy's fixative may preserve mast cells best, it does cause tissue distortion as best exemplified by red cells which become ghosts and it also destroys immunological activity. It is not possible therefore to combine immunoperoxidase techniques with Carnoy's fixation. Fixation with aqueous buffered formaldehyde (formol) preserves tissues better than Carnoy's and the tissues retain most of their antigenicity. The detection of IgE by immunoperoxidase techniques produce a brown colour on the cell surface. Morphologically it may be difficult to differentiate mast cells from plasma cells. Azure A counterstains mast cell granules metachromatically from blue to violet. The contrast does not work well with immunoperoxidase techniques where the light brown colour may be swamped. Toluidine blue may be used in dilute solution

and purple metacromasia fades quickly so permits a combination of these two techniques.

The purpose of this section is to see whether fixation demonstrates two obvious subpopulations of cells, to combine immunoperoxidase with toluidine blue to see the number of mast cells with IgG_4 and IgE on their surface and finally to document the ultrastructure details in normal nasal tissue and see if there is any evidence for mast cell degranulation in polyp tissue and to see if it extends further into the rest of the nasal mucosa.

MATERIALS AND METHODS

Intranasal preparation

The following method of preparation was used for all specimens. Preoperatively the nose was treated with cocaine solution 5% followed 10 minutes later by cocaine paste 25%.. The paste was applied topically to the mucosa, and to the sphenopalatine and anterior ethmoidal nerves as nerve blocks if the surgery were performed under local anaesthesia. Polyps were removed by an avulsion snare and the intraethmoidal tag was removed. The inferior turbinate was removed with either nasal turbinectomy scissors or biopsied with Tilley-Hinkel forceps when studied. Material was always removed from the same side.

Routine histology was performed when the polyps were unilateral to exclude malignancy.

Fixation

Ten patients were studied and selected at random from the routine waiting list. Polyps were placed for either buffered formol saline which

was used for routine histology (Hopwood 1982) or into Carnoy's fixative. Carnoy's fixative is absolute ethanol 60%, chloroform 30% and glacial acetic acid 10% (Hopwood 1982). Similarly the inferior turbinate biopsy was divided into two and placed in either formol saline or Carnoy's fixative. Specimens were coded randomly A, B,C or D. in the laboratory and the code was broken after evaluation.

Tissues were processed routinely by a paraffin schedule for tissue fixed in aqueous formaldehyde or into absolute ethyl alcohol for those samples in Carnoy's fixative. All specimens were blocked into paraffin wax. Sections of 4μm were cut and stained with Azure A. Mast cell granules stain metachromatically from blue to purple. The number of cells in ten random high power field were counted separately both within the epithelium and interstitium for each specimen.

Statistical analysis

Since the results contained zeros and some large numbers the distribution was positively skewed. The median values and ranges were used to compare groups. The distribution was evaluated by non parametric analysis using the Friedman test. There were eight categories for each patient made up of four samples with distribution into either the epithelium or interstitium. The number of cells was rated from 1 to 8 for each patient with a mean of 4.5.

Immunoperoxidase for IgE on polyp mast cells

Fourteen patients were studied. Six had positive skin tests and three of these had hay fever. Two of the hay fever sufferers also had asthma and asthma was present in a further case with negative skin

tests. Using the widest criteria there were six atopic or possibly atopic patients. All were adult males with ages from 29 to 81.

Tissue was removed as previously described and processed through a paraffin schedule. Sections were cut at 4 μm. Consecutive sections were counterstained with haemotoxylin and eosin, and toluidine blue. Photographs were taken using Kodak 135 film.

Immunoperoxidase technique

The unlabelled antibody method was used with prior trypsination as described by Sternberger, Hardy, Cuculis, Meyer (1970). Endogenous peroxide is blocked by 3% hydrogen peroxide. Affinity purified antihuman IgE 2mg/ml (Ishizaka) was diluted 1:500 with this buffered saline. 10 μl of human serum (1 in 10) was added to every ml to remove non specific activity. Pig antirabbit serum (Dako) was used and combined with rabbit peroxidase antiperoxidase (Dako).

The same method was used for IgG except that sheep antihuman IgG raised by the animal research centre at Babraham (Z511G) was substituted for antihuman IgE and goat antisheep and sheep peroxidase antiperoxidase complexes were used (both Dako) for the further steps.

Controls using normal rabbit and sheep instead of the antihuman immunoglubulins were also prepared.

Toluidine blue solution was prepared by diluting 50 ml of 0.1% toluidine blue with 50 ml of distilled water containing 3 mg of ferrous sulphate which was filtered prior to use.

Nuclei stain dark blue with haematoxylin and light blue with toluidine blue. Mast cells stained metachromatically light purple and immunoglobulins stained brown. Cells were examined without reference to

the clinical history. It became obvious that no simple classification was possible since specimens tended to be similar.

Electron microscopy

Normal nose: The inferior turbinate was trimmed from five patients who were admitted for septoplasty, submucous resection or septorhinoplasty. All operations were performed for nasal obstruction following trauma, none of the patients had a history of nasal disease including allergic, vasomotor or viral rhinitis. Apart from blockage due to the deflected septum, the patients had no other symptoms and had negative skin tests. Surgery was performed under general anaesthesia with cocaine nasal preparation. The turbinate was trimmed because of physiologial hypertrophy in the unobstructed (normal) side.

Nasal polyps: Fifteen patients with nasal polyps were examined. Pieces one millimeter thick and half a centimeter long were cut off the nasal portion of the polyp. Similar sized biopsies of the inferior turbinate on the same side were taken.

Tissue preparation: The material was diced into cubes of approximately one millimeter and as much of the epithelium as possible was included. These were placed into 2.5% glutareldehyde in cacodylate buffer within 5 minutes of surgery and fixed for 4 hours. The fixative was initially at $4^{o}C$ and allowed to come up to room temperature. Material was placed into fixative within 5 minutes of removal. Tissue was then placed into buffer for 1 hour and subsequently washed in distilled and deionised water. The blocks were stained with uranyl

acetate for half an hour and dehydration was carried out through an acetone series. The blocks were infiltrated with Spurr resin (Spurr,1969). Semithin sections of 1 μm were taken for staining with toluidine blue, using a hot plate. Ultrathin sections of 90 ηm were mounted on a copper grid and were double stained with uranyl acetate and lead citrate (Reynolds 1963). Sections were examined on a JEOL 100S transmission electron microscope and photographs were taken on Kodak 4489 EM film.

Histological examination: Routine examination for the detection of mast cells was abandoned since mast cells were frequently found on electron microscopy which were not detectable at the light microscope.

Electron microscopy: Between 3 and 20 blocks were examined from each biopsy. At least 5 mast cells with nuclei were seen and photographed for each person. Electron microscopy is descriptive and results were not easy to quantify to overcome this only cells with nuclei were photographed. Representative findings are presented here.

Degree of degranulation: 20 cm by 25 cm photographs of whole mast cells were placed under a transparent sheet divided into squares of sides 1 cm or 1 in. The number of granules in the cell was counted and the number of partially or completely empty ones noted. The degree of degranulation was expressed as a percentage of all the cells counted. Some degranulation is found in normal cells and based on results from normal patients any patient with polyps with a figure above 75% of the granules intact was considered normal. The results were grouped into

four categories, (1) above 75%, normal or minor degranulation; (2) between 75% and 50%, moderate degranulation; (3) between 50% and 25% and (4) below 25%. Each patient's cells were evaluated and grouped accordingly.

RESULTS

Fixation: It became apparent that the background staining was more intense using formol fixation and this was obvious despite the blind nature of the study. It was also possible to differentiate inferior turbinate from polyp because the former contains glands and a much more extensive vasculature. Mast cells were found distributed mainly in the connective tissue of all specimens and the numbers from ten comparative high power fields were significantly higher in the sub mucosa for all specimens with either method of fixation, Friedman's test, $P<0.01$). The median and ranges of the number of ten random high powered fields are given below.

	FORMOL		CARNOY'S	
	Polyp	Turbinate	Polyps	Turbinate
Epithelium	0 (0-5)	0 (0-0)	9 (4-19)	3 (0-122)
Connective tissue	18 (4-66)	15 (3-80)	30 (11-72)	32 (2-130)

These results suggest that human mast cells cannot be characterised by site using their fixation charactistics. Mast cell morphology was varied though most cells were circular about one in ten was spindle shaped. The position of the nucleus within the cell also varied from central to the side.

Numbers of cells within the polyp and turbinate tend to be slightly less in formol fixed tissue and this could result either from fixation

characteristics of either the tissues or the mast cells since it was easy to notice the difference in background staining between the two methods. Carnoy's fixative allows mast cells to stand out more clearly and this may well account for the difference in the numbers.

IgE and IgG on mast cells

Five patients had fewer mast cells than the remaining patients but they all confirmed to the distribution noticed above. No mast cells were seen coated with anti IgG. Mast cells coated with anti IgE were encountered in all polyps and approximately half the mast cells had no obvious IgE on their suface. Some mast cells appeared to have more IgE on their suface. It was difficult to assess whether the IgE coated mast cells were more or less degranulated since it was easier to see the IgE on the less granulated cells. Since immunoperoxidase staining was variable, it was difficult to make accurate assessment of the degree of staining. There was no difference between those patients with atopy and those without.

IgG and IgE plasma cells were encountered and their staining was much more intense than the mast cells.

Two mast cells are shown below,(Figs 2.1 and 2.2) unfortunately the light purple of the granules has been lost in the main from the photographs taken from the transparencies. The original magnification of both is 320. The first picture shows a much more intense staining by the immunoperoxidase. The granules are seen slightly better in the second but has little IgE on the surface. Both pictures were taken from the same histological slide.

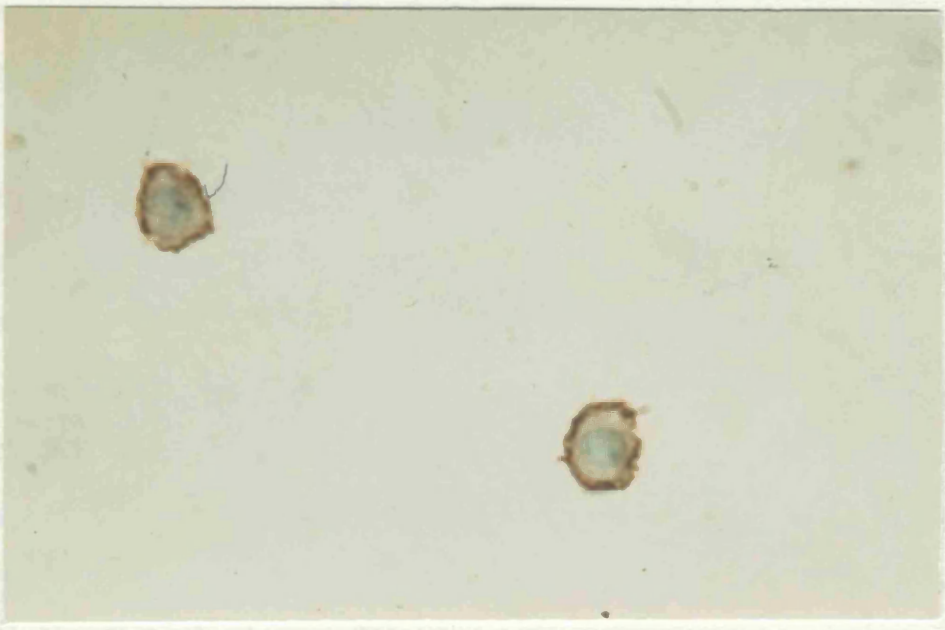


Figure 2.1

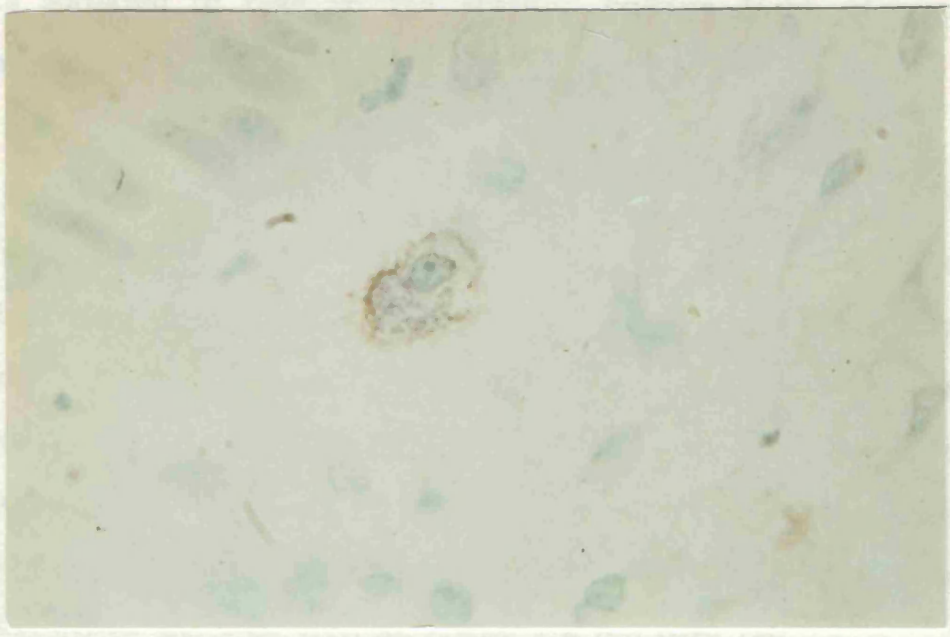


Figure 2.2

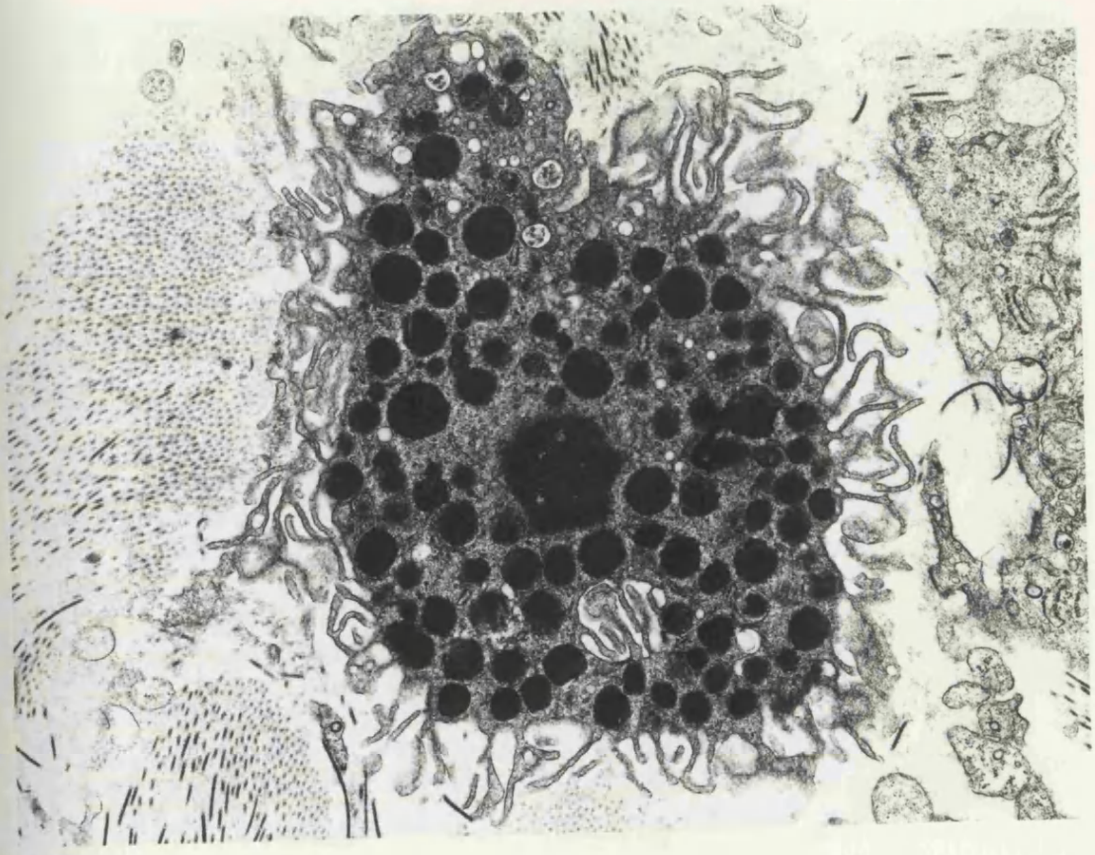
Ultrastructure of normal nasal mast cells

Representative mast cells of the five normals which were encountered are shown in six figures (2.3-2.8). Figures 2.3 and 2.4 come from the same patient and Figure 2.7 contains the most obvious degree of degranulation (arrowed).

Mast cells were rarely encountered within the epithelium and were scattered patchily through at the submucosal tissue. Sometimes up to three blocks had to be examined before mast cells were encountered and then two or three were seen in close proximity. They could be seen near all types of structure including glandular structures, nerves and blood vessels. There were no obvious prediliction for any structure. In general the distribution confirmed the findings of light microscopy.

Light microscopy on EM fixed tissue counterstained with toluidine blue was of little use for predicting mast cells since only the larger cells appeared to take up the stain. Routine light microscopy was abandoned.

Shape and size: Mast cell size and shape was variable. About one in ten were spindle shaped. The majority were circular, some of the degree of difference may be seen in the six electron micrographs. Figure 2.4 shows an elongated cell with a 'tail'. The difference in morphology makes it difficult to classify cells on size. Some cells are obviously larger and others smaller. In general a spectrum of cell sizes and shapes exist and the size varies from 10-20μ. The larger cells had more granules and some such as Figure 2.4 had very few.



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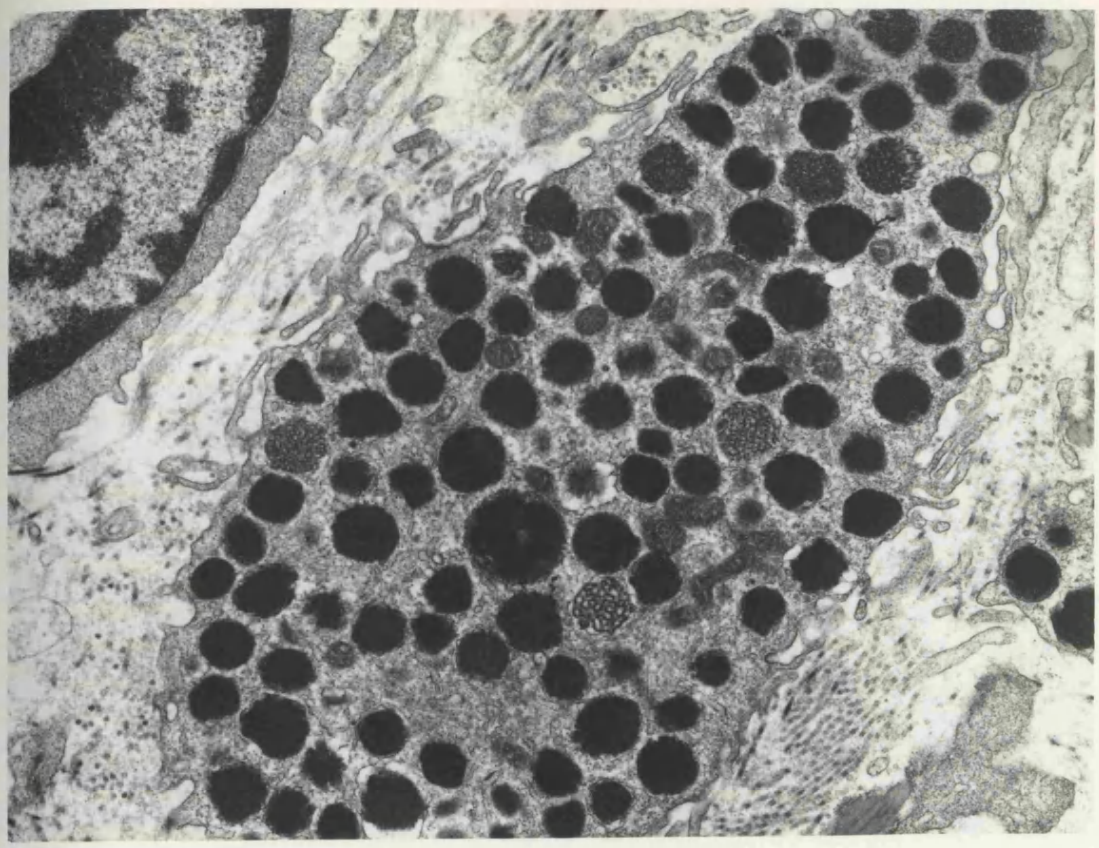

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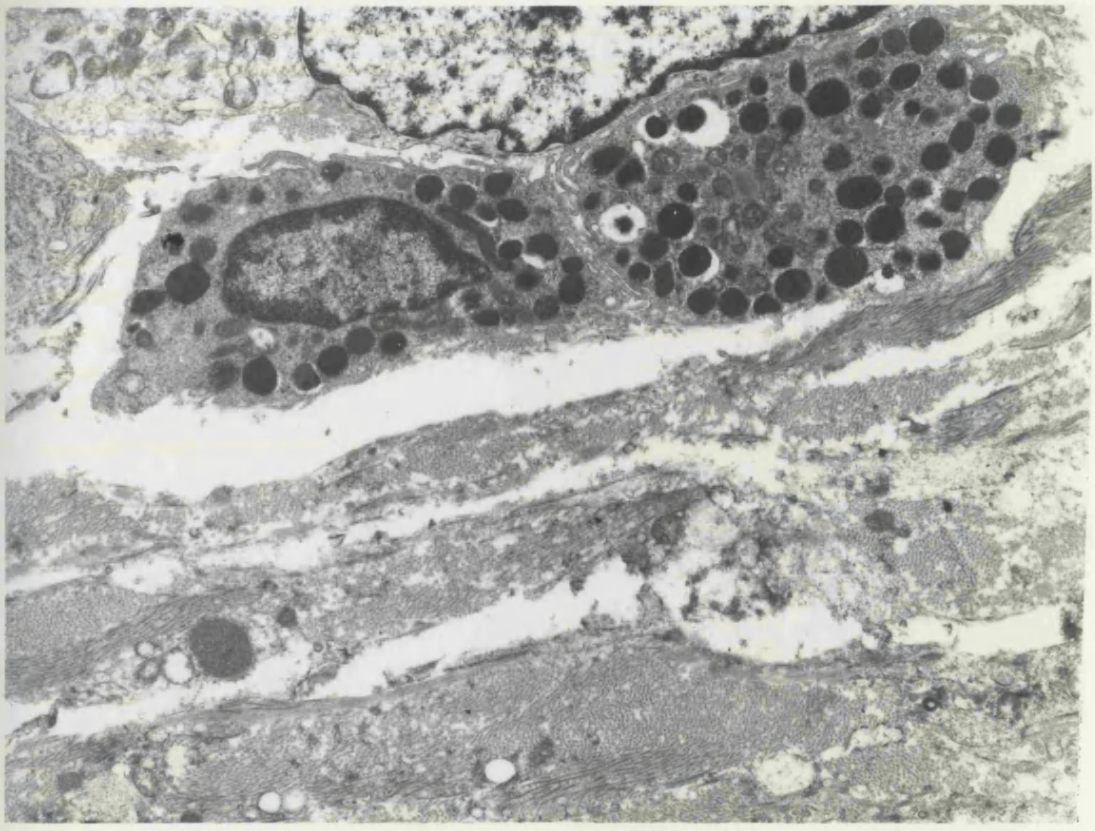

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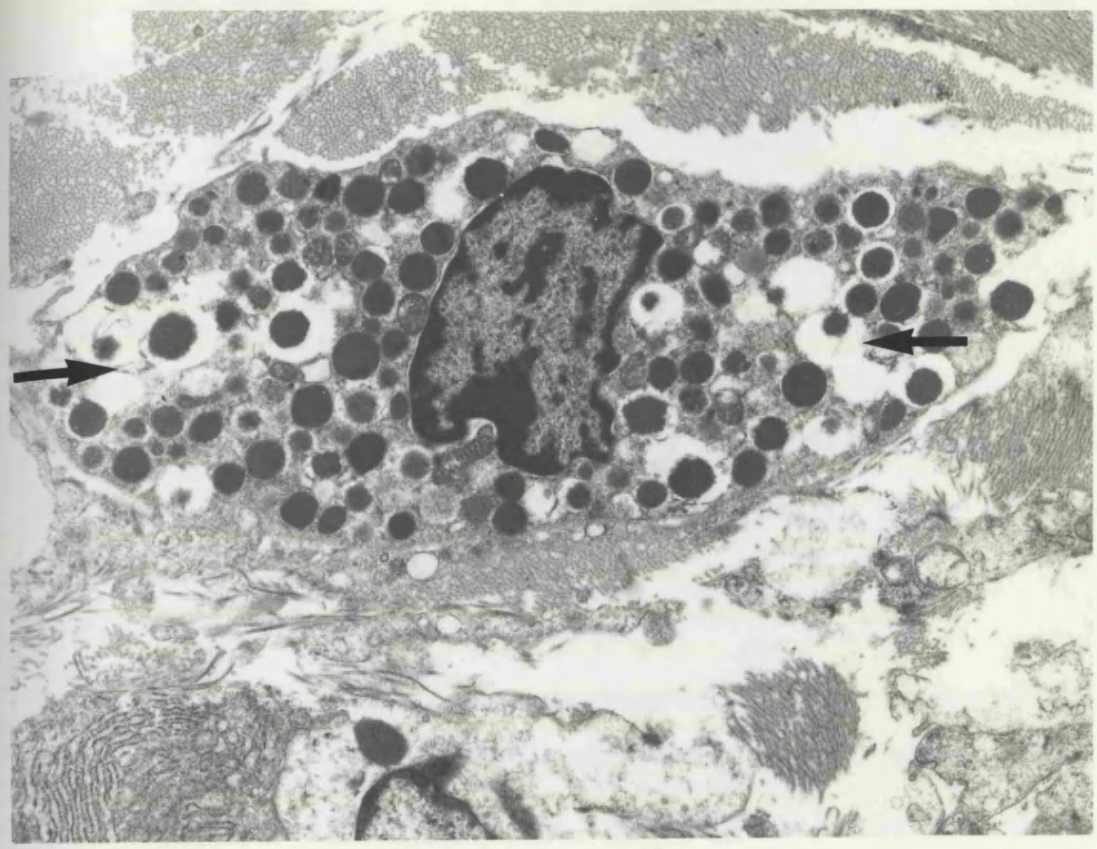
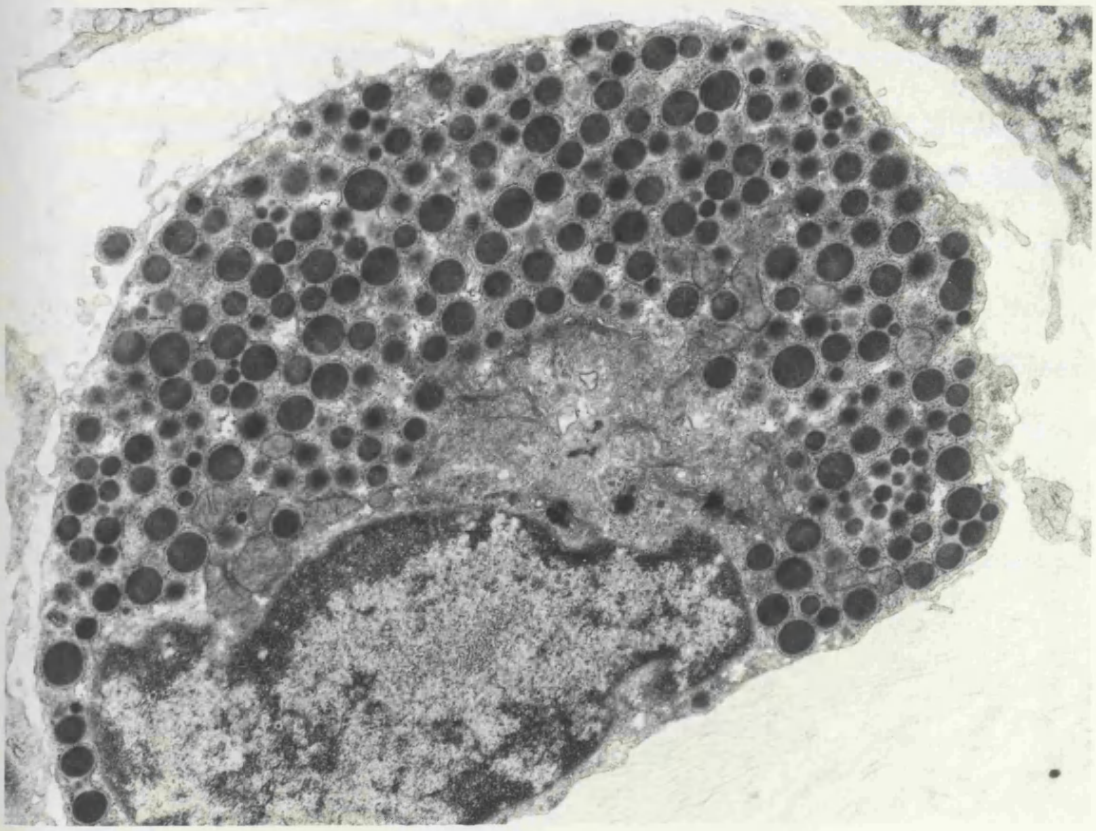


2.7



2.8

Surface projections: Figure 2.3 shows a cell with a large number of projections whereas Figures 2.6 to 2.8 have very few. A few were normally encountered in every cell and their size was variable. They contained cytoplasm and are similar to the projections seen in macrophages.

Nucleus: A single circular nucleus which varied in position was found. Chromatin was electron dense around the edge of the nucleus as is seen in Figures, 2.3-2.8. This type of nucleus helped to identify degranulated mast cells in polyps.

Cytoplasm: All cells contained mitochondria. There were no constant features including position, numbers and shapes. A few cells had many more than others although all the cells shown here had similar numbers and would appear typical.

Golgi apparatus: This was seen in some cells and is particularly well developed in Figure 2.8.

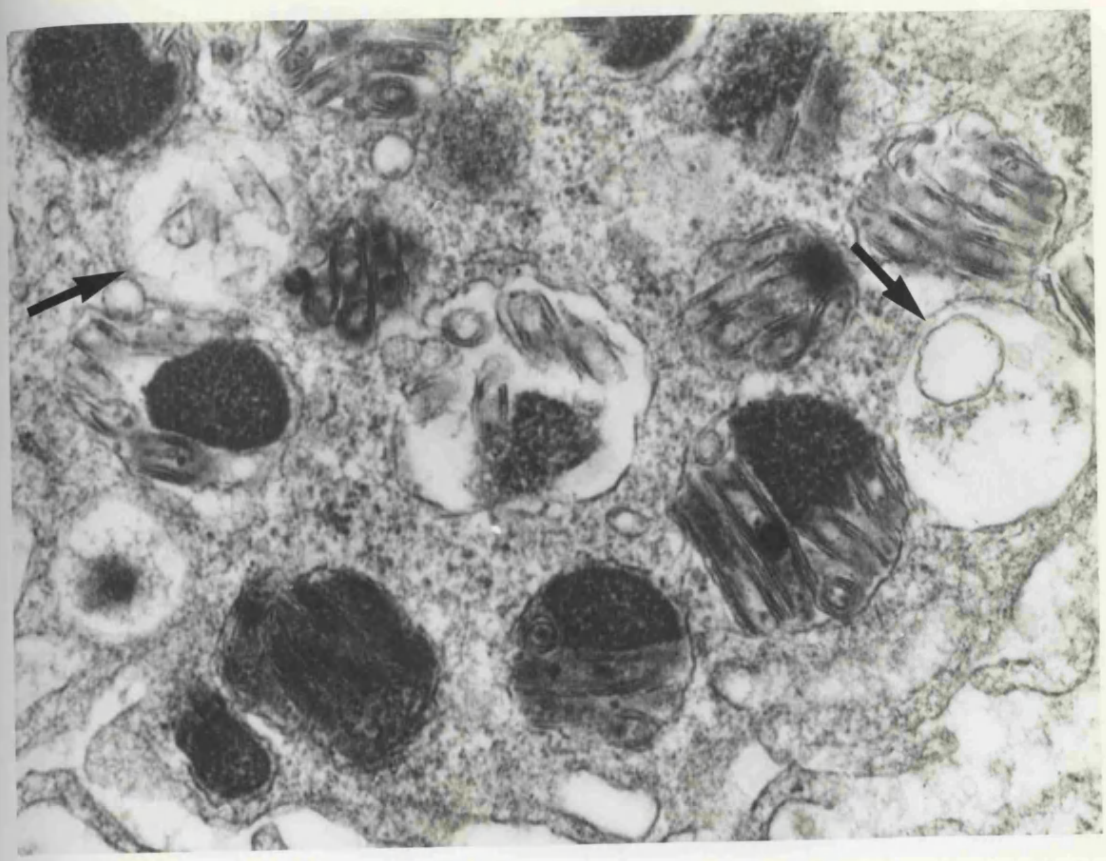
Lipid bodies: They were unusual and were seen more commonly in pathological material as is demonstrated in Figure 2.14 (L).

Granules: The most striking feature of the normal mast cell which is electron dense. The number of granules varies as is seen in the first six figures. Even at this magnification it is possible to see that most of the granules were electron dense and amorphous. Granules were distributed evenly through at the cytoplasm.

Figures 2.9 to 2.12 show the variation of granule morphology. When further details were visible, scrolls were encountered most frequently. Figure 2.9 shows a mixture of amorphous granules and scrolls. Two vacuoles are present (arrowed). They were called scrolls because they resemble parchment scrolls (Fig 2.10), when when they were cut along their long axis they appeared as tubes. The ghost of a scroll may be the only feature to identify a degranulated mast cell. Granules can contain more than one structure. Figure 2.11 shows several 'multi granules'.

A crystalline matrix is the other distinctive feature and is rarely seen in nasal tissue. It is hexagonal and when cut along its long axis appears as a series of parallel lines (Fig 2.12). Figure 2.12 also shows a patchy electron dense granule which may sometimes appear as a tapeworm as in Figure 2.5. This structure was seen infrequently in normal nasal tissue but was often encountered in polyp mast cells.

There was some degree of degranulation in normal cells such as Figure 2.7 and it was never more than 25% of the total granule numbers. It was more difficult to assess whether different ultrastructural features were granules in the process of releasing their contents so that if there were a clear margin around less dense material or over half a normal granule were empty, then the granule was considered to be degranulating. Some shrinkage artifact can occur in normal cells which is why this criteria was used with electron dense material. A vacuole is the end stage. In view of these findings pathological tissue containing cells with more than 75% of their granules intact were considered as normal tissue.



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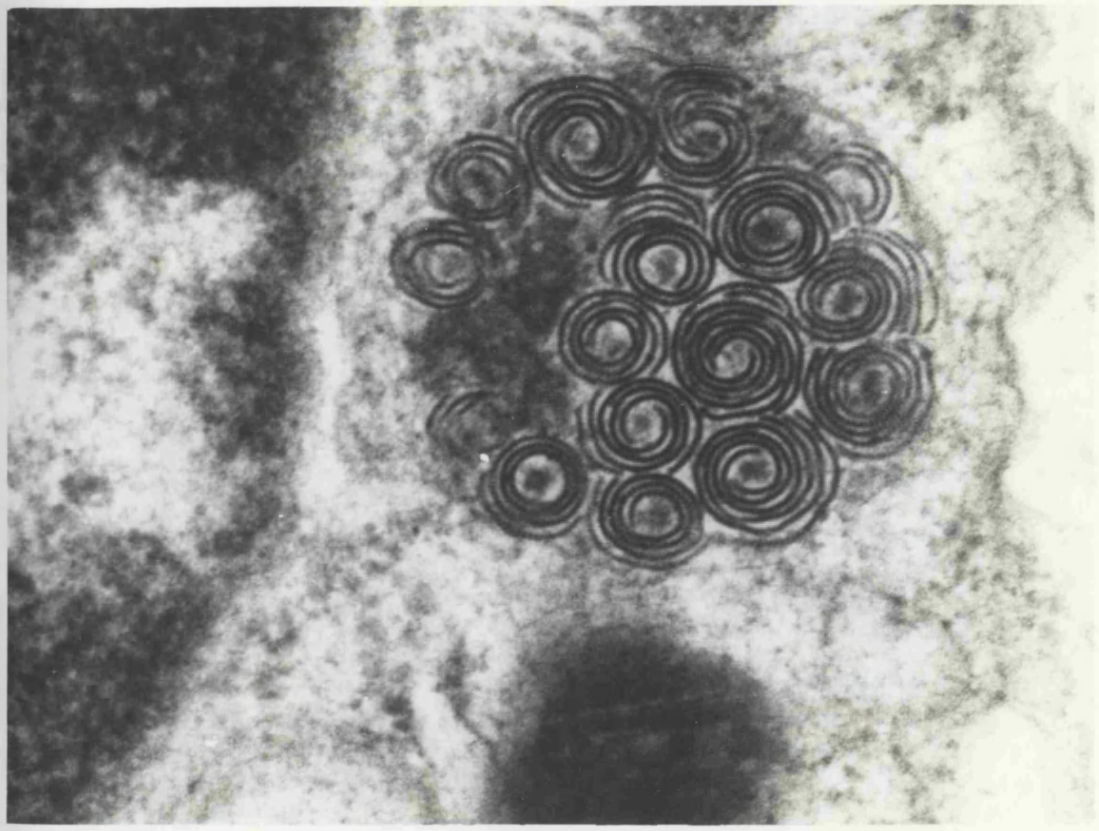

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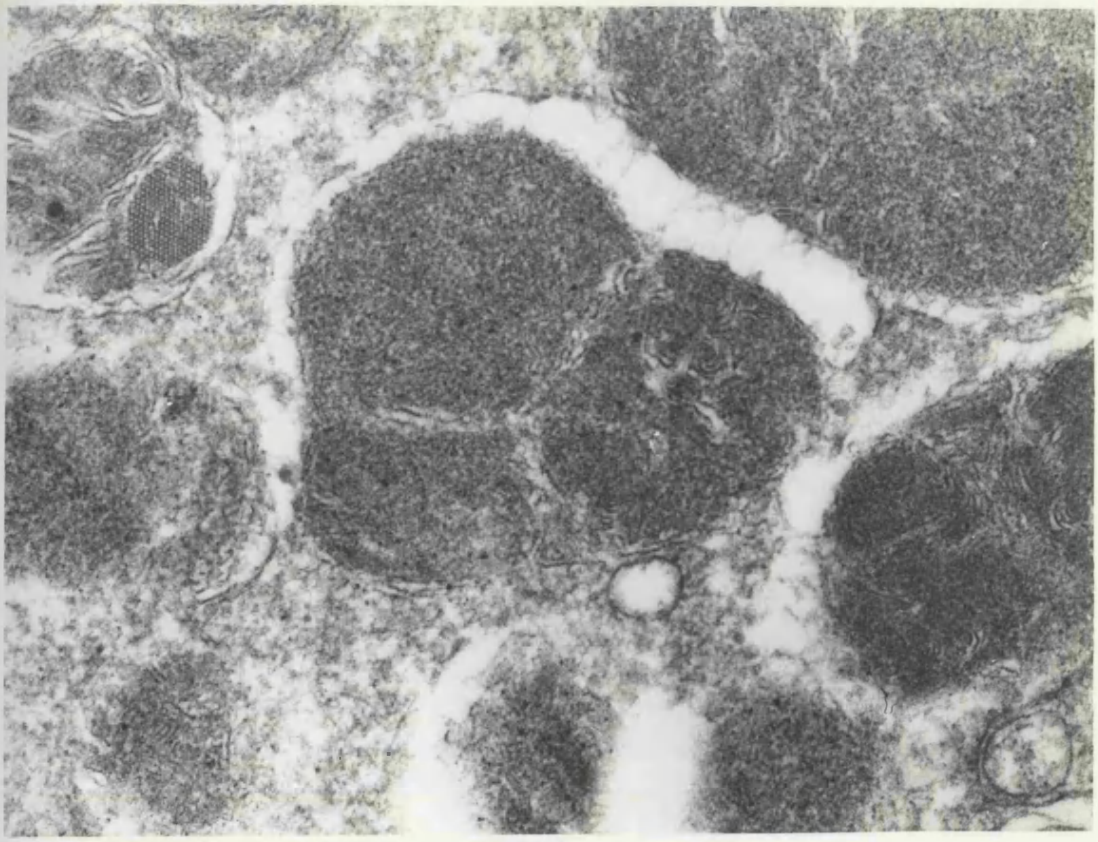

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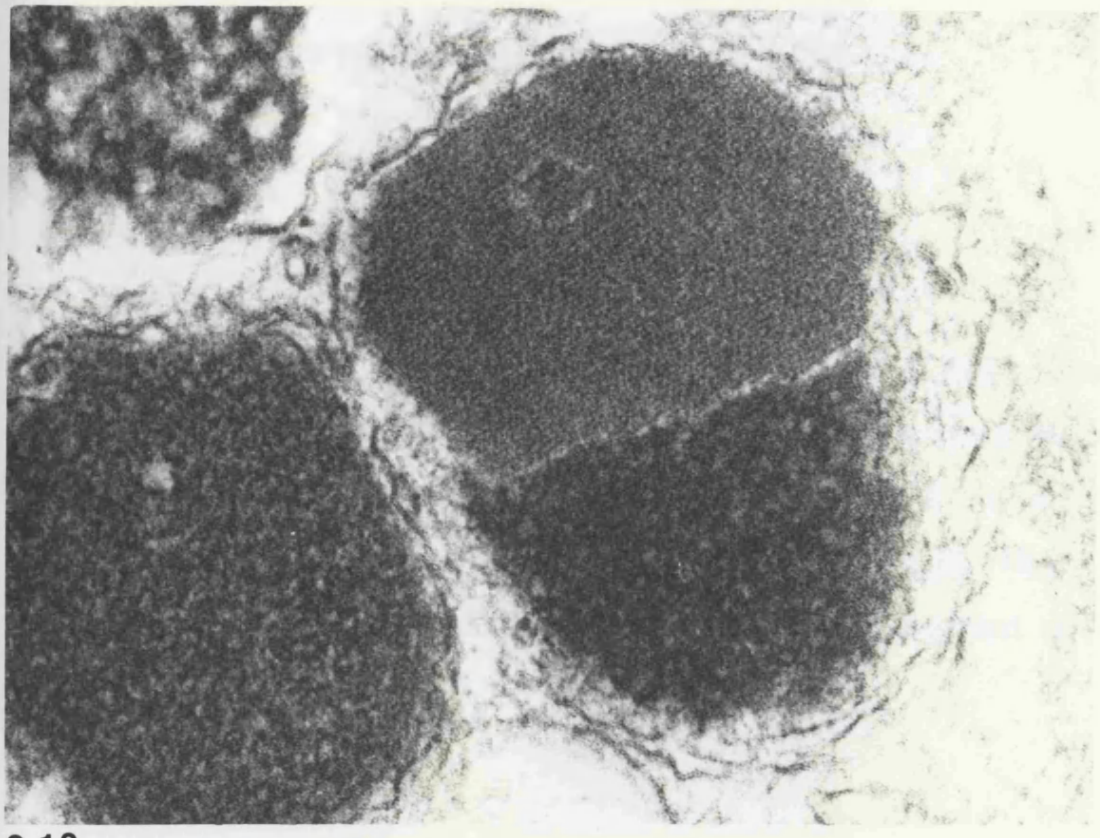


2.12

Polyp mast cells

Two mast cells (Figs 2.13, 2.15) are shown and demonstrate that virtually all the normal granule morphology had altered. It is possible to make out the remnants of scrolls in Figure 2.13. The following figure shows more extensive changes. The condition of the inferior turbinates was much more variable (Figs 2.14, 2.16). Four patients had normal morphology and a further four had stage 2 degranulation. Two patients had extensive degranulation and in five the cells were almost completely empty of electron dense material.

Degranulation of Mast Cells in Polyps and Turbinates

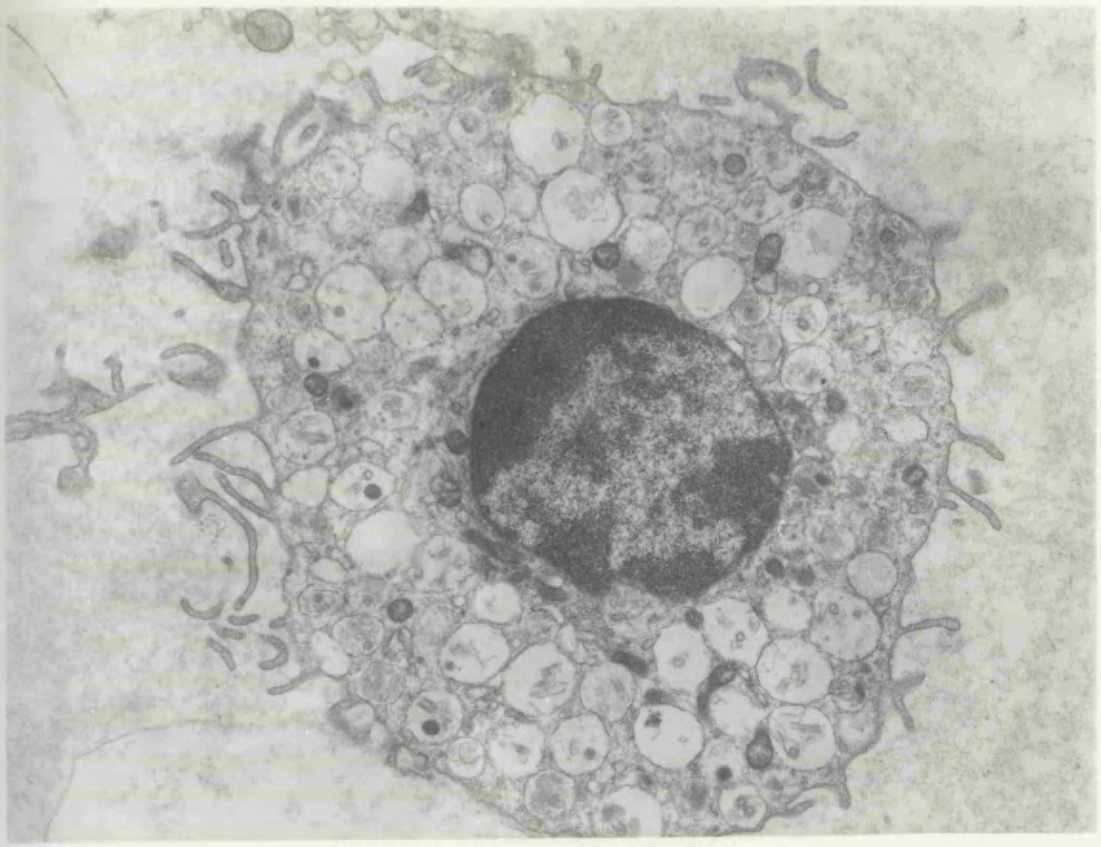
Normal granules		Polyp	Turbinate
100-75%	(1)	0	4
75-50%	(2)	1	4
50-25%	(3)	4	2
25%	(4)	10	5

This group included four women and three asthmatics.

DISCUSSION

Fixation.

Mast cells are difficult cells to study because of their fixation characteristics and staining properties. When they were first described by Erhlich he noted that water based fixatives were less reliable than alcohol based compounds (1879). These difficulties have been excellently reviewed by Michels (1938). Michels also pointed out that



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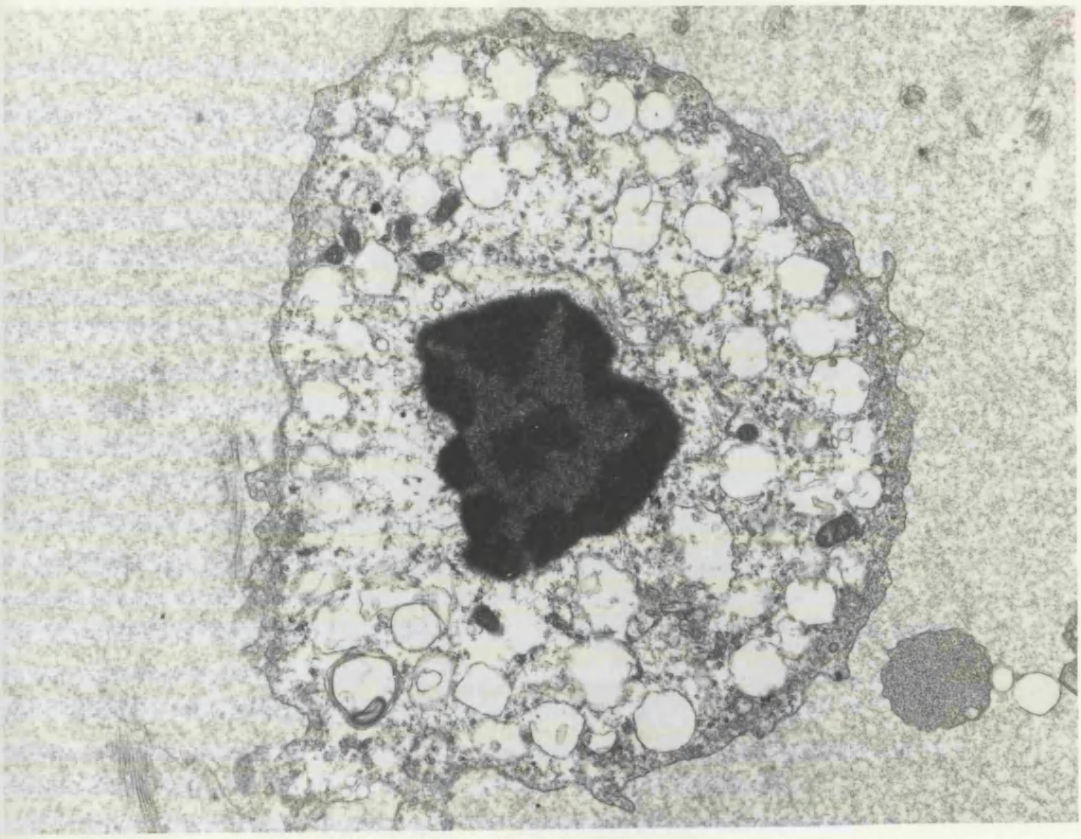

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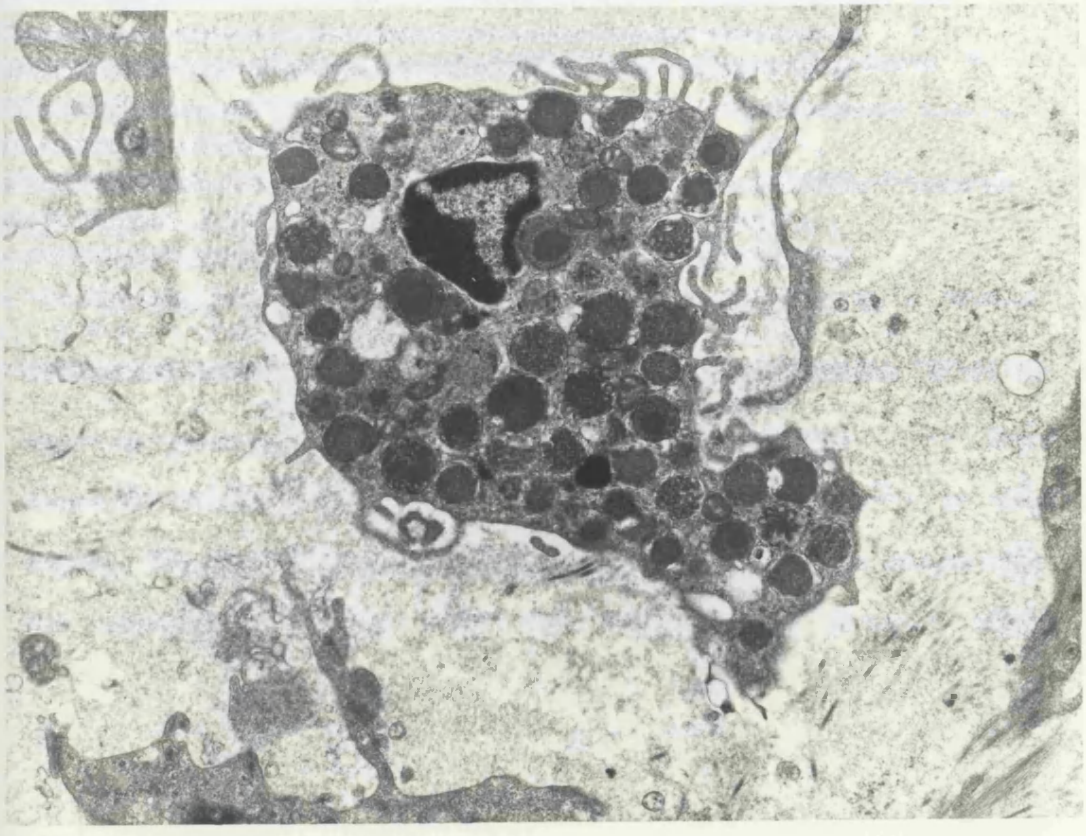


2.16

acetic acid may also change granule morphology and this is used in Carnoy's fixative. In general the granules are difficult to demonstrate consistently. The granules were previously considered 'water soluble' but they are quite stable in the aqueous medium of the body and may be shown on frozen section. Frozen sections have limitations when applied to routine histology since they are labour intensive and because of ice crystal formation do not show tissues as well as routine fixation and blocking. More recently long formaldehyde fixation has gathered support since granule morphology became more distinct with time in rats (Wingren and Enerback,1983).

The differences in mast cells to fixation has been used to classify mast cells in animals into connective tissue and mucosal mast cells. There are three groups of basophilic metachromatically cells in theory. The two mast cell types and the circulating basophils. The rat has very few circulating basophils and two mast cell types are easily demonstrable in the tissue (Michels 1938). The guinea pig has all three cell types and the rabbit large numbers of basophils and fewer mast cells. There has been little work done on man. One study which looked at the fixation characteristics of the human small bowel suggested that there may be two types of mast cells (Strobel, Miller, Ferguson, 1981). There have been no comparable studies on nasal mucosa.

The first part of this section was to demonstrate a difference in mast cells within the epithelia and connective tissues which could be shown by fixation properties of different cell types. Mast cells were shown better by Carnoy's fixation for reasons that will be considered later. The distribution within the epithelium and the connective tissue was markedly different with few cells being present within the

epitheleum. Mast cells have been found in large numbers within the surface epithelium during the hay fever season in sufferers (Viegas et al,1987) and during the winter months this is reversed. The lack of epithelial mast cells found here argues against any acute allergic response in patients with polyps. This study does not confirm the impression that there are two populations of mast cells within the nose demonstrable by fixation differences. The same may not apply to the gut (Strobel, Miller, Ferguson, 1981).

Mast cells are much easier to detect using Carnoys fixative and this is mainly due to background staining. Carnoys fixative is not used for routine work in laboratories since it does not preserve tissues well and gives rise to technical difficulties with other stains. Consequently the background drops back and therefore mast cells stand out. Preservation was much better with formaldehyde but mast cells are more difficult to identify because background detail was more intrusive. This may account for some of the differences in mast cell numbers encountered in the two fixation methods.

There was no obvious difference between the polyps and the inferior turbinate.

Immunoperoxidase

Immunoglobulins have been found in nasal secretions and in the normal nasal mucosa (Brandtzag et al 1967). Histofluorescence has been used to look at the distribution of plasma cells within polyps (Bass et al 1974, Nakashima and Hamashima 1980).

Bass et al used polyps and inferior turbinate from ten patients. They prepared routine sections with formalin fixation and stained

section with haematoxylin and eosin and toluidine blue and also prepared immunofluorescent sections from frozen tissue. They checked specificity of the anti IgA, G and M on renal biopsy material and anti IgE on tonsillar tissue.

Although they comment mainly on IgA and IgG their findings on IgE plasma cells are of interest. There was no staining for IgE in the submucosa and only two patients had IgE plasma cells in polyp tissue and they were scanty. They also stated that they found no mast cells in polyp tissue on routine staining. An exceptional finding which casts doubt on the reliability of their findings.

Nakashima and Hamashima (1980) looked at the polyps from thirteen patients and used fluorescent staining of paraffin embedded sections. They used a direct method to detect IgA and IgG which is unusual and the more common indirect method for IgM and IgE. They made no comments on the distribution of IgE and confined their comments to IgA.

Another histofluorescent study (Whiteside et al 1975) examined plasma cells from fifteen patients and used a commercial IgE. They divided their patients into atopic, doubtfully atopic and non atopic. They called patients with hay fever, asthma, whatever the cause, and patients with positive skin tests as atopics. Not only were the categories they used questionable but the results from the small numbers were not able to stand up to their conclusions that there were allergic and non allergic polyps.

Histofluorescence is complicated and it is difficult to counterstain to define cell types. Although it is possible to stain sequential sections, none of the studies discussed here mentioned this and did not appear to stain for mast cells. Fluorescence staining fades and it is

not possible to check results later.

The results in this study showed that the amount of IgE on the surface of the mast cell was very variable both within polyps and from patient to patient. There was no obvious difference between patients who were grossly atopic compared with those who were not.

Light microscopy demonstrates mast cell granules by two properties, metachromasia and enzyme activity. Metachromasia is produced when blue cationic dyes react with acid groups in a structure which cause polymerisation of the dye and metachromasia to occur. This is usually to transmit light of a shorter wave length eg from blue to purple. Enzyme methods use substrates which hydrolyse carboxylic acids. The commonest substrate used for mast cells is napthol AS-D chloroacitrate esterase but unfortunately it is not specific for mast cells and demonstrates myeloid cells. The only specific substitute is naphthol AS aminocaproate (Li, Lam and Yam 1973), but this is not available commercially.

Electron Microscopy

Studies of cells at the light level should be confirmed ultrastructurally. Tissues are both fixed and stained differently. Electron microscopy uses water based fixatives such as buffered gluteraldehyde, the commonest buffer used here was cacodylate which is a sodium arsenate. Fixation is rapid and penetrates only a short distance. The stain used for orientation is toluidine blue, which fortuitously stains mast cells metachromatically. However Galli et al (1984) stated that light staining is of little value in detecting mast cells before electron microscopy. This was confirmed here and is not

related to the ulstrastructural morphology since the granules are similar in all cell types. The property of cells used in electron microscopy is the ability of compounds to bind heavy metal salts of osmium and lead.

The normal mast cell granule when examined electron microscopically is electron dense. The ultrastructure may be organised or may be amorphous. Although dissociated human lung mast cell granules are organised into scrolls or crystalline patterns, normal nasal mast cell granules are usually amorphous but may show both these features.

If the shape and size are used to categorise mast cells then this study showed that the normal cell varied considerably in size, shape, number of surface projections, distribution of mitochrondria and the presence of golgi apparatus. Granules showed a number of changes associated with degranulation even in the normal nose, but it was never extensive.

Rodents release their granules direct into the extracellular fluid (Kessler and Kuhn 1975, Dvorak et al 1983). Occasionally the granules may dissolve prior to release as is the norm in humans. Dissociated pulmonary mast cells which have scrolls as the commonest intragranular feature show the following sequence of in vitro changes when challenged with anti IgE (Caulfield et al 1980). The process is rapid and is completed within 20 minutes. The granules enlarge and become amorphous, then either by the formation of a microtubular system or by direct contact between the granules and the surface membrane of the cell, the contents are discharged into the extracellular fluid. A vacuole results and there is no obvious matrix outside the cell. There has been one study using biopsies of nasal tissue (Trotter and Orr 1978).

Unfortunately the paper gave no details of either the number of patients studied nor any clinical details and also included tissue from the lower respiratory tract. There were no normal data for comparison. They described a process similar to that which was described in in vitro lung tissue.

In vivo challenge of the nose by allergen has been studied by Kawabori and his colleagues (Kawabori and Unno 1983) in five patients who had perennial rhinitis. They suggested that the features differ and the changes occurred more superficially near the surface.

Nasal secretions have been studied by Okuda et al (1983). They suggested that most of the allergic reactions occurred on or above the epithelium and were mediated by basophils. Nasal secretions are difficult to examine cytologically and quantitative examination is not possible. Autolyses and degeneration are often seen (Wihl 1979). This casts some doubt on the validity of Okuda's study.

Previous studies on electron microscopy on polyps have commented on the mast cells (Cauna et al 1972, Busuttil et al 1976). They noted that they were degranulated but although documenting it this was ignored by Cauna and his colleagues who considered that the prime pathology was neurovascular.

There is little doubt that all mast cells in polyp tissue were degranulated most markedly irrespective of the age or history of the patient. The changes extended throughout the submucosa and mast cells were rarely seen in the epithelium which argues against allergy. This was also found on light microscopy.

In two thirds of the patients, mast cell degranulation was also found in the inferior turbinate, which gives support to the hypothesis

that mast cell reactions may be more extensive than within the polyps and fit with clinical findings of rhinitis in a proportion of cases prior to the development of polyps.

In conclusion mast cell degranulation has been found ultrastructuraly in all polyps and this may extend throughout the nose. Different fixations showed that the distribution of mast cells was constant with fewer cells in the epithelium. This argues against allergy as the likely cause and only half the mast cells were found to be covered with IgE even in patients with a history of allergy.

The next section will examine the nature of polyp fluid and confirm that it is oedema and that the levels of histamine present are consistent with mast cell degranulation. Levels of allergen specific IgE and IgG_4 in house dust mite and mixed grass pollens will be evaluated to see if the commonest allergens may be implicated in the mast cell degranulation.

PART THREE

EVALUATION OF POLYP OEDEMA

FREE HISTAMINE IN POLYP FLUID

Clinical material

The histamine levels in polyp extracellular fluid were measured in fifty two patients. Thirteen patients had a history of asthma, four of these had aspirin hypersensitivity, five had a history of hay fever and twenty two had positive skin test results to one or more allergens. Polypectomy was performed as previously described.

Polypectomy

The nose was prepared as before and polyps were removed. Polyps were placed immediately onto a gauze swab moistened with isotonic saline. This was to minimise the transudation of fluid which readily occurs. The polyps were then washed in isotonic saline to remove local anaesthetic, mucus and blood. They were then placed into a plastic pot containing a gauze swab moistened with physiological saline. The polyps together with 10 mls of clotted blood were transferred to the laboratory for immediate extraction of polyp fluid and serum. The polyp fluid was extracted by coarsely slicing the polyps with scalpels and microfuging at 12,000 rpm for 5 minutes. A straw-coloured fluid was obtained from the polyps and was stored at -20^{o}C until analysed. Serum was prepared in the standard way.

Histamine analysis

Serum and polyp fluid diluted one in ten initially with PBS 0.15 M pH 7.2, were deproteinised by the addition of an equal volume of 0.8M perchloric acid. After centrifuging at 4,500 rpm for 10 minutes the clear supernatant was decanted and assayed spectofluoremetrically for

histamine using a Technicon Auto Analyser Industrial Method No 164-73E which is based on the methods of Evans et al,1973. Three patients had their polyps removed without anaesthetic, the histamine levels, although not included here, showed significant free histamine 130, 1,800 and 2,240 ng/ml. Both cocaine and adrenaline solutions as used in local anaesthesia did not give any interference in the histamine assay. It was not possible to obtain a satisfactory solution of cocaine paste since the paste is paraffin based and not water soluble.

TOTAL PROTEIN

50μl of blank, sample and standard were pipetted into 5 ml of working biuret reagent (Technicon Chemical Co). The samples were mixed and allowed to stand at room temperature for 20 minutes. They were then read on a calorimeter fitted with a 555 nm filter. There was a linear relationship between 20 and 150 gm/litre in serum (Doumas,1975). The blank was distilled water and the standard was a reference serum (Technicon Chemical Co) both for the total protein and albumin levels.

ALBUMIN

25μl of blank, sample and standard were pipetted into 10 mls of working bromocresol green dye reagent (Technicon Chemical Co). The samples were mixed by inversion and read in a calorimeter fitted with a 625 nm filter. The results are linear up to 60 gm/l of albumin (Doumas, Watson and Biggs,1971).

ELECTROPHORESIS

4μl of sample were electrophoresed in 1% agarose gel (Miles Research Products) made up in barbitone buffer 75 m mols/l at pH 8.6. This was supported on a polyester sheet. Electrophoresis was performed at 250 v constant voltage for 45 minutes. The plate was fixed in picric acid, 15 gm/l for 15 minutes, dried and then stained with coamassic blue R250 (Sigma, Poole) (Jeppsson, Laurel and Franzen,1979).

IMMUNOGLOBULIN CONTENT OF NASAL POLYP FLUID

MATERIALS AND METHODS

Clinical material

Twenty-nine adult patients with nasal polyps were studied. They included six patients with asthma of which one had aspirin hypersensitivity, one had hay fever and eleven had positive skin tests to one or more allergens.

Nasal polypectomy and the preparation of polyp fluid were carried out as previously described.

Immunoglobulin content

The results from the previous three experiments showed that the protein content in nasal polyp is similar in distribution to the serum but it is slightly less in amount. The immunoglobulin measured in nasal polyp fluid may arise either from local plasma cell production or be present due to vascular leakage. If the level of immunoglobulin is below that of serum it is difficult to be certain if there is significant local production. In order to overcome this difficulty the level of protein not synthesised locally but present only by passive

diffusion can be compared both in polyp fluid and serum to the levels of immunoglobulin both in polyp fluid and serum. These values may be expressed as a ratio (Donovan et al,1970). They measured both albumen and α_2 macroglobulin (α^2m). Albumin is present in such quantities and the method used here was so sensitive (reverse passive haemagglutination) that it was difficult to obtain a satisfactory end point. The previous section shows that both large and small molecular weight proteins leak equally through the capillary basement membrane so that α_2m, which is comparable in weight to IgM, would be sufficient to give an indication of the amount of protein and immunoglobulin present by diffusion.

An index of local production in polyp fluid may be found by comparing the ratios of immunoglobulin (IgI) andα_2m:

$$\frac{\text{IgI polyp}}{\text{IgI serum}} \text{ divided by } \frac{\alpha_2\text{m polyp}}{\alpha_2\text{m serum}}$$

Reverse passive haemagglutination is performed using serial dilutions and in this case two-fold ones. It is possible to express the ratio as a logarithm to the base two:

($\log_2$ IgI polyp - log IgI serum) + ($\log_2\alpha_2$m serum - $\log_2\alpha_2$m polyp) Rather than calculating the actual value and taking the log, it is much easier to consider the numerical value of the end point well, (end point IgI polyp - end point IgI serum) + (end pointα_2m serum - end pointα_2m polyp), eg (7 - 8) + (10 - 7) = 2. This is called the local production number LPN). A positive number indicates significant local production even if the level of immunoglobulin in polyp fluid is less than that in serum. Zero or negative numbers indicate that the majority may have

arisen by diffusion.

Reverse passive haemagglutination

Animal antiglobulin linked to an indicator red cell is a sensitive method of detecting proteins to which the antiglobulin has been raised. The antiglobulin is coupled to a tanned red cell and the method used here was the piperazine method which is a modification of the standard chromic chloride method (Scott et al,1981). Human red blood cells group O (Human RBC), stored in acid dextrose citrate at 4°C were used as the indicator cells. Cells were washed six times in phosphate buffered saline pH 7.2 (PBS) and a 10% suspension in PBS was mixed with an equal volume of 0.25% trypsin at pH 7.0 (Difco). The mixture was incubated for 30 minutes at 37°C. This was then centrifuged and the cells washed twice in PBS. The cells were resuspended and mixed in an equal volume of 0.025% trypsin inhibitor (Sigma), they were then left for 10 minutes at room temperature. Sheep antihuman IgG, IgA, IgM, IgD, α_2m and normal sheep IgG, together with rabbit antihuman IgE and normal rabbit IgG at a dilution of 2 mg/ml were individually coupled with the treated blood cells (IgG, Sheep 2511 Cr; IgA, Sheep 2649 V; IgM, Sheep 2631 V; IgD, Sheep 2316 Cr; M, Sheep 2401 T; all kindly supplied by the University of Birmingham. The rabbit anti-human IgE, of affinity purified rabbit antihuman IgE, was a personal gift to Professor Coombs by Professor Ishizaka). A 0.15 mg/ml solution of chromic chloride in 0.9% NaCl was titrated with an 0.27 m solution of piperazine hydrate in N HCl until the pH was 6.5. This was mixed with an equal volume of 0.9m NaCl to produce the working buffered solution. Three volumes of this buffer were mixed with one volume of trypsin treated red cells and one volume

of animal immunoglobulin. Two volumes of 0.15 mg/ml chromic chloride were added slowly during mixing. This was mixed further for an hour at room temperature. Cells were then washed three times in PBS and resuspended in a 1% solution in PBS. The preparations were checked for auto agglutination and specificity against pure human IgG, IgA and IgM.

Standards

IgG, IgA and IgM standard serum 67/86 was used throughout and the sensitivity of IgG was 1.22×10^{-4} U/ml; of IgA $\times 10^{-3}$ U/ml; and of IgM 3.125×10^{-2} U/ml. IgD standard serum 67/37 had a sensitivity of 3.125×10^{-2} U/ml and IgE standard serum 75/502 had a sensitivity of 0.39 U/mll (National Biological Standards, London). The sensitivity of IgE measured by this method is comparable to that of the PRIST method (Scott et al,1982).

Titrations

Polyp fluids and sera were used undiluted for IgE estimations and a stock one in 40 dilution of each match polyp fluid and serum, 25μL made up to 1 ml in PBS was used throughout for all other titrations. All titrations were serial two-fold dilutions with matched fluids and sera. IgM, IgD andα_2m titrations were performed in the 1 in 40 stock whereas IgA and IgG started at a dilution of 1 in 1600 (by diluting the 1 in 40 stock by 10 and then 4). The end points were confirmed by either Professor RRA Coombs or Dr ML Scott.

Controls

Human RBC coupled with N sheep or rabbit of globulin were run

against all the dilutions of sera and polyp fluid. Anti sheep activity was found in 11 polyp fluids and sera but in no case did it cause any difficulty in estimating the end point. Anti rabbit activity was found in 8 polyp fluids and 10 sera; again no difficulty was found in estimating the end point.

Statistical Analysis

The IgE in polyp fluid was divided into values below and above 400 U/ml and the serum below and above 200 U/ml. All the clinical parameters were compared with polyp fluid and serum IgE levels by chi squared tables with suitable corrections for small numbers and Fisher's Exact Test, when the latter was applicable.

RAST LEVELS TO HOUSE DUST MITE AND MIXED GRASS POLLENS IN POLYP FLUIDS AND SERA

MATERIALS AND METHODS

Polyp fluid, together with matched sera from twenty eight patients was used to perform RAST (radioallergosorbent tests). The method here was to bind the allergen to cellulose filter paper (Ceska,1972). Labelled antihuman IgE and pooled atopic sera were kindly provided by Dr M Kemeny (Department of Medicine, Guy's Hospital).

Allergen Discs

Cyanogen bromide (CNBr) activated cellulose filter paper discs were prepared by cutting the filter paper into 6 mm circles which were then washed with 2L of distilled water. Twenty five grams of CNBr were

dissolved into 300 ML of distilled water overnight in the fume cupboard. The CNBr solution was added to the drained discs and the pH was adjusted to 1.0 by addition of 1 M NaOH. This was mixed with a spatula for 10 minutes. The solution was poured off and inactivated with 200 ml of 15% Sodium Hypochlorite. The discs were then washed with 5L of 0.05 M $NaHCO_3$ at 4°C followed by 0.5L of acetone also at 4°C. They were then freeze dried overnight.

Coupling of Allergens to Discs

One gram of activated discs were placed in a 30 ml universal container (x 2). 10 ml of the allergen extract (Dephonolised HDM and MGP extract, Bencard) in 10 ml of 0.1 m $NaHCO_3$ were added and mixed for 24 hours at 4°C. The discs were then washed in 0.1 m $NaHCO_3$ at 4°C for 10 minutes and then twice in 0.1 m B-ethanolamine in 0.1 m NaCU, at a pH of 8.0 by adding NHC1 for one hour at 4°C, and twice in sodium acetate pH 4.0 for 10 minutes at 4°C. Finally the discs were washed twice in PBS and then distilled water for 10 minutes at 4°C.

Assay

Twenty eight sera and polyp fluids together with four normal sera were prepared by putting 50μl of test sample into 450μl of 50% human serum albumen. 50μl of this was pipetted into four Luckham LP3 tubes and two tubes had one disc of HDM and two had one disc of MGP added. These were incubated overnight and then washed five times with 2 ml of PBS containing 0.1% Tween 20. 50 1 of anti-IgE 1^{125} (50,000 mcpm/50μl) was added to each specimen. Two tubes acting as a negative control both had 50 μl of 1^{125} anti IgE alone were incubated overnight and then

washed five times as before.

Standard Curve

Pooled highly allergic sera nominal 1000 units was diluted 1 in 5, 1 in 10, 1:20 in 50, 1 in 100, 1:200 in 500 and 1 in 1000 in 50% human albumen.

Counts

Counts were measured on a Wallac Decem series gamma counter for one minute.

LEVELS OF TOTAL HDM AND MGP IgG4 IN POLYP FLUIDS AND SERA

Patients

Thirty seven patients were studied. Eight of the thirty seven patients studied had asthma and two of these had aspirin sensitivity. Three patients had hay fever and one of these had asthma in addition. Skin tests were positive in a total of sixteen patients and only three were positive to both HDM and MGP together (Table 3.4). Eleven patients had twelve operations during the period May to August inclusive which is considered here as the hay fever season.

Polypectomy was performed under local anaesthetic of cocaine paste as previously described and the oedema fluid together with a matching serum sample was stored at -20°C until analysed.

Samples 1 and 38 are from the same patient; he had two polypectomies separated by a year; his results were considered together. Patient 36 had dermatographia and so his skin tests were excluded from analysis.

Allergen-specific and total IgG ELISA

The wells of microtitre plates (Falcon, Microtest III, Becton-Dickinson), were coated by overnight incubation at 4°C with 125μl of either house dust mite (D.pteronyssinus extract, code 4050, from Bencard) diluted 1/10, mixed grass pollens (Allpyral 5 grass mix, 10,000 PNU/ml, from Dome Labs) diluted 1/50 or sheep anti-human IgG_4 antiserum, diluted 1/500 in 0.05M carbonate buffer (pH 9.6).

Sensitised plates were washed (x 3) in phosphate buffered saline (50 mM phosphate, 0.9% NaCl) containing 0.05% Tween 20 (r/r) (PBS/T). Standard sera dilutions and test serum samples, diluted 1/20 for allergen-specific IgG_4 and 1/50 for total IgG_4, 100μl samples were added to each well in duplicate and plates incubated for 2 hours at room temperature in a moist chamber. After washing, as above, a mouse monoclonal anti-human IgG_4 antibody (clone RJ described by Lowe et al,1982) diluted 1/1000 in PBS/T, was added (100μl/well) and the plates re-incubated, then washed, as before. 100μL of goat anti-mouse IgG/horse radish peroxidase conjugate (Nordic), diluted 1,2000 in PBS/T, was then added to each well and the plates re-incubated and washed, as before. Finally, 100μL of freshly prepared substrate (orthophenylene diamene dihydrochloride, 0.4 mg/ml, from Sigma, and hydrogen peroxide 0.6% w/v from BDH in distilled water) was added to each plate. The reaction was stopped by adding 2 L of 4N H_2SO_4 to each well and the optic density (OD) of each well at 492 nm was read using an automated plate reader (Multiskan MC, Flow Labs, Irvine, Scotland) interfaced to a BBC micro-computer (Acorn Model B). The substrate background OD was subtracted from all serum values.

The standard IgG_4 serum was doubly diluted, from 20.0 μg/ml IgG_4

(1/10 initial dilution) and a standard curve constructed by plotting mean OD values against concentration (Layton and Stanworth,1984). IgG_4 levels in test sera (diluted 1.50) were found by interpolation of each OD value from the standard curve and expressed as means of the duplicates in μg/ml. Allergen-specific IgG_4 standard sera were also doubly diluted from 100% (1/5 dilution) and test tesults expressed as percentage standard (% standard), with no correction for dilution of test samples.

RESULTS

Free Histamine

The histamine levels ranged from 124 to 7,300 ng/ml with an arithmetic mean value of 1,700 ng/ml (Table 3.1). From the results this mean value would appear to be biased towards the high end. This corresponds well with the median value of 1,055 ng/ml. For the purposes of statistical analysis levels below 1,000 ng/ml were taken as low values and levels above as high values. Parameters were compared using Chi-squared tables with suitable corrections for small numbers. There were no significant differences between the polyp histamine levels in those with a history of asthma, aspirin hypersensitivity, hay fever and positive skin tests.

The corresponding sera were between 2 and 20 ng/ml with a mean of 10 ng/ml. These results are high because wherever possible the same dilutions were used and the histofluorentric analysis was at the lower end of sensitivity for the sera.

Table 3.1:

Study No.	Polyp histamine level	Serum histamine level	Study No.	Polyp histamine level	Serum histamine level
13	124	12	26	1,070	12
16	150	12	48	1,080	8
6	252	4	39	1,160	10
12	270	12	45	1,280	10
65	335	16	44	1,400	12
21	340	12	40	1,450	8
70	352	10	71	1,460	14
9	370	4	22	1,520	12
19	380	12	73	1,600	6
5	413	12	41	1,880	10
32	420	6	30	1,960	6
64	450	10	10	2,080	4
27	530	10	63	2,225	7
47	620	14	46	2,300	12
69	704	8	68	2,560	10
50	720	10	72	2,640	14
35	750	4	28	2,700	4
18	790	18	60	2,900	20
25	820	4	17	2,926	16
59	820	6	58	4,100	6
24	840	12	34	4,320	8
38	860	16	36	4,500	4
14	910	16	45	4,800	10
31	1,000	4	65	4,900	16
67	1,000	10	62	6,700	7
61	1,040	9	8	7,300	4

Protein

Table 3.2 shows the total protein and albumin levels for paired nasal polyp fluids and sera. In all cases the amount of protein and albumin was less in polyp fluid. The mean value of total protein was 60 gm/l for polyp fluid (range 52 - 69 gm/l SD 4.69) compared with 69.6 gm/l for serum (range 65 - 76 gm/l SD 2.65). Similarly, the albumin mean value in polyp fluid was 33 gm/l (range 29 - 38 SD 3.25) and in

serum was 39.9 gm/l (range 36 - 44 SD 2.36). Polyp fluid has significantly less protein and albumin than serum ($P < 0.001$ students t test)

Table 3.2

	Total protein g/l (Manual Biuret)	Albumin g/l (short reacting BCG) (on IL Multistat III)
5 NP	58	32
S	68	38
19 NP	69	36
S	76	44
32 NP	60	30
S	69	40
44 NP	60	38
S	68	41
45 NP	62	36
S	65	37
46 NP	59	31
S	72	39
47 NP	52	29
S	68	38
48 NP	60	32
S	71	42

Electrophoresis

The results are shown in Figures 3.1 and 3.2. In both polyp fluid and sera all the major electrophoretic groups, pre-albumin, albumin, α_1, α_2, and γ globulins were present. There appeared to be no gross differences between the matched pairs.

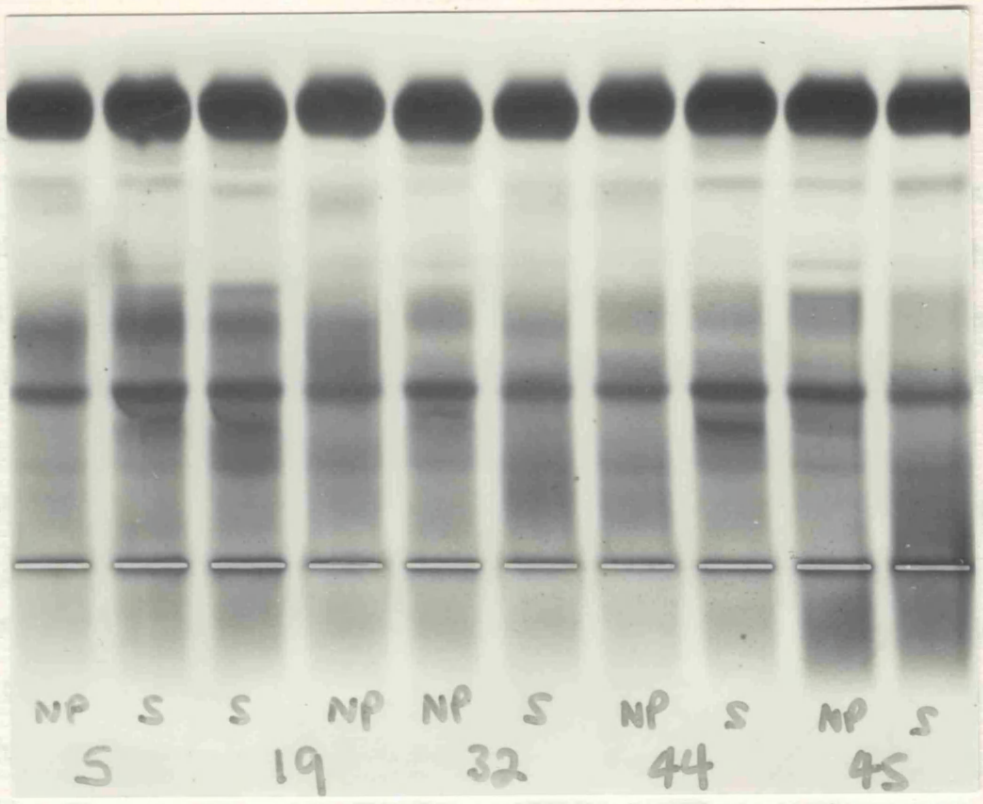


Figure 3.1

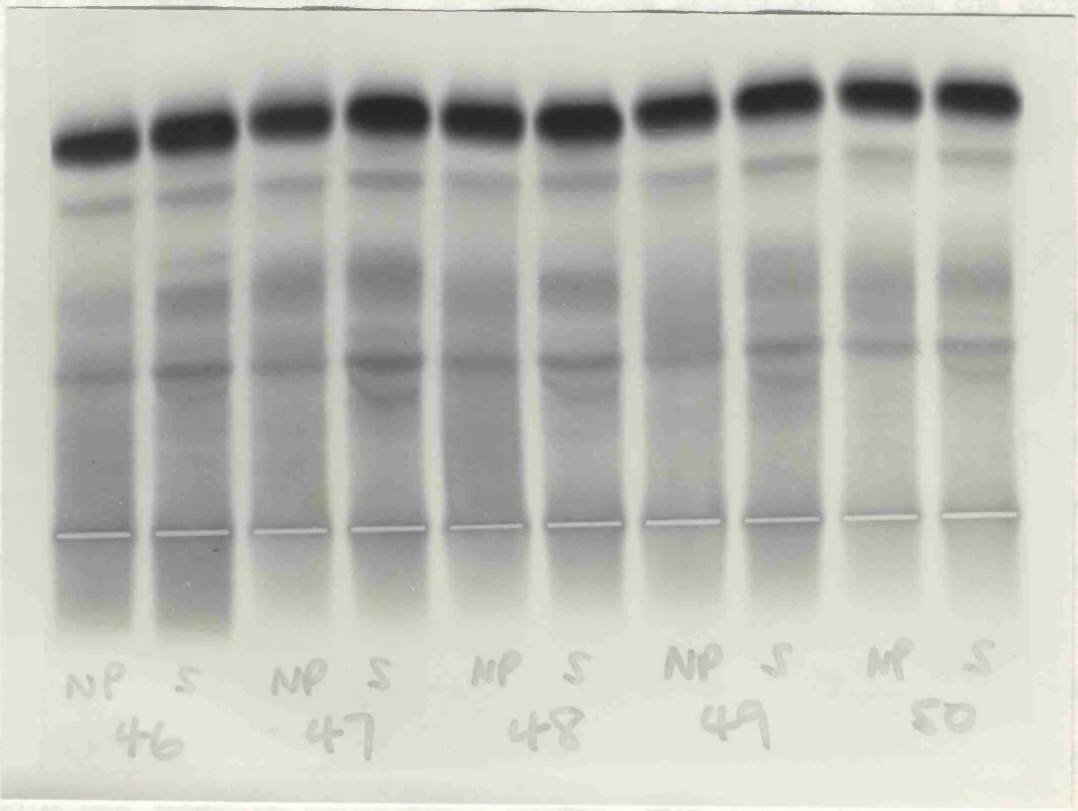


Figure 3.2

Immunoglobulins

The concentrations of IgG, IgA, IgM, IgD and IgE which were found in matched polyp fluids (P) and sera (S) are shown in Table 3.3. The end points of titrations for α_2m are also shown in this table. The level of α_2m was lower in polyp fluid in twenty eight cases and the same in only one. This confirms the finding that the protein content of polyp fluid is less than that of serum and is consistent with the previous results.

IgG. The concentration of IgG in matched polyp fluids and sera showed that in only one case was the level greater in polyp fluid; it was the same in ten cases and lower in eighteen. It would appear that diffusion could account for considerable quantities of IgG in polyp fluid. The mean in polyp fluid was 100 U/ml and that of serum was 200 U/ml; the range was similar in both fluids (Fig 3.3). When the effect of dilution and leakage was accounted for, the LPN is negative or zero in eleven patients and positive in eighteen and confirms significant diffusion in at least eleven cases (Fig 3.4).

IgA. In thirteen cases the levels of IgA were higher in polyp fluid; also in thirteen cases the levels were the same and in only three cases were the levels lower in polyp fluid. The mean was 400 U/ml in polyp fluid and 200 U/ml in serum; the ranges were similar in both fluids and sera. The LPN was zero in two cases and positive in twenty seven cases, suggesting that there was appreciable local production of IgA in all cases but two (Figs 3.3 and 3.4).

IgM. In four cases only was the level of IgM higher in polyp fluids, it was the same in fourteen and lower in eleven patients. The mean value was lower in polyp fluid 80 U/ml than serum 160 U/ml. These results were reflected in the LPN; it was zero in five cases and

positive in twenty four.

IgD. The mean IgD in both polyp fluids and sera was the same, 160 U/ml. The paired values were higher in polyp fluid in 16 patients, the same in nine and lower in four. The range was wide in both polyp fluids and sera. The mean used was arithmetic although whether this was correct is not clear. The LPN was positive in all but one case.

IgE. The distribution of IgE was skew and it is the accepted practice to use the geometric mean. Polyp concentrations lay between 12.5 and 3,200 U/ml with a mean of 400 U/ml. The serum values lay between 6.25 and 3,200 U/ml with a mean of 100 U/ml. The paired polyp fluids and sera had lower values in polyp fluids in two cases, equal in five and above in twenty two. The LPN was positive in all cases.

Distribution

Although reverse passive haemagglutination is a sensitive assay the technique tends to allow variation of the end point so that results will vary by one tube either side of the actual value. Both IgG and IgA are present in considerable amounts and both in polyp fluids and sera appear normal in distribution (Fig 3.3). The range of values was close for both IgG and IgA with the majority one measurement above and below the mean. Only one value for IgG in polyp fluid and serum was two measurements lower or higher than the mean (3%). Similarly, for IgA only one value was two tubes lower in polyp fluids (3%) and one above and below in sera (7%). This would suggest that the wide range of values for IgM, IgD and IgE was real and that comment may be made concerning their distribution. IgM had a normal or Gaussian distribution both in polyp fluids and sera. The distribution both of

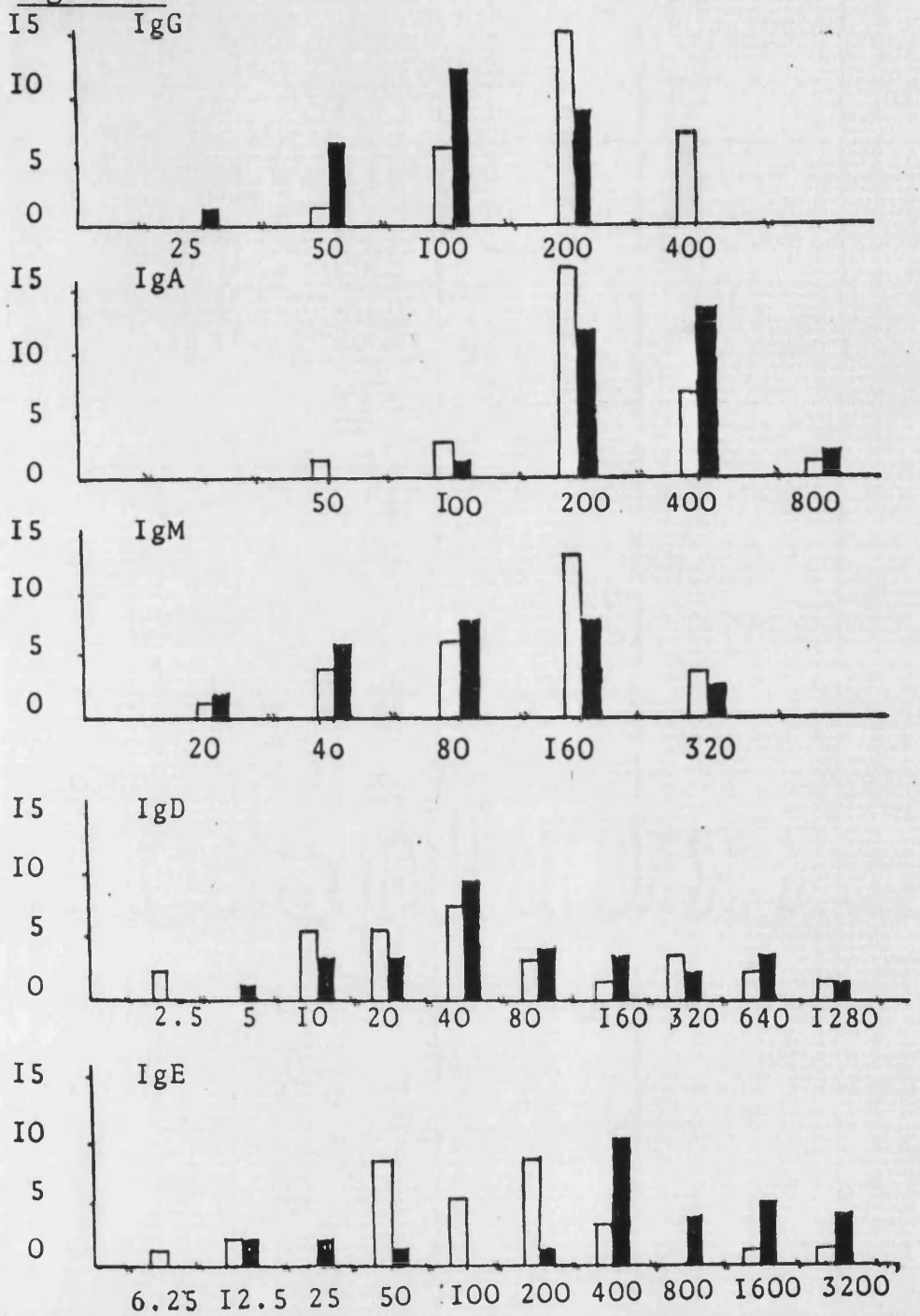
IgD and IgE was very wide and skew. IgE in polyp fluids would appear to fall into a high group (24) and a low group (5), which were below 100 U/ml.

Table 3.3 Immunoglobulin levels in nasal polyp fluids and sera

No.	IgG		IgA		IgM		IgD		IgE		α_2M*	
	P	S	P	S	P	S	P	S	P	S	P	S
5	25	50	400	200	320	160	40	10	200	50	7	8
6	100	200	400	200	40	160	1280	640	12.5	12.5	6	8
8	100	100	200	100	160	160	160	40	800	50	8	9
9	50	200	200	400	40	20	10	2.5	400	100	5	8
10	50	200	400	200	160	160	160	80	3200	400	7	9
12	50	200	400	200	160	160	80	320	400	200	6	9
13	50	200	200	200	40	40	40	20	12.5	6.25	7	8
14	100	100	400	400	80	80	40	20	1600	100	7	8
16	200	400	200	200	80	320	80	80	1600	200	6	8
17	100	200	200	200	80	80	20	20	400	100	5	7
18	50	100	200	50	20	80	160	40	1600	200	6	9
19	50	200	200	200	40	160	40	10	400	200	7	9
21	200	200	400	200	80	80	10	10	400	50	7	9
22	200	100	100	100	160	320	40	40	25	50	7	9
24	100	400	400	400	40	40	5	2.5	400	100	7	8
25	100	100	400	400	20	40	20	10	25	50	7	9
26	100	100	400	400	160	160	80	40	3200	200	7	7
27	200	200	400	200	80	80	640	320	400	50	8	9
28	200	200	400	200	80	160	40	20	400	200	6	8
30	200	200	400	200	40	80	20	40	800	50	8	10
31	50	200	200	200	80	40	80	80	400	400	8	10
32	200	200	800	200	80	80	40	10	800	200	8	9
34	100	400	400	400	40	160	320	1280	3200	1600	7	9
35	100	400	400	100	80	160	40	40	400	400	8	9
36	100	200	800	400	320	180	640	160	1600	12.5	9	10
38	100	400	200	200	320	320	320	320	3200	3200	9	10
39	100	400	200	800	160	320	40	40	400	100	7	10
40	200	400	200	400	160	160	10	20	800	200	8	10
41	200	200	200	200	160	160	640	640	50	50	9	11

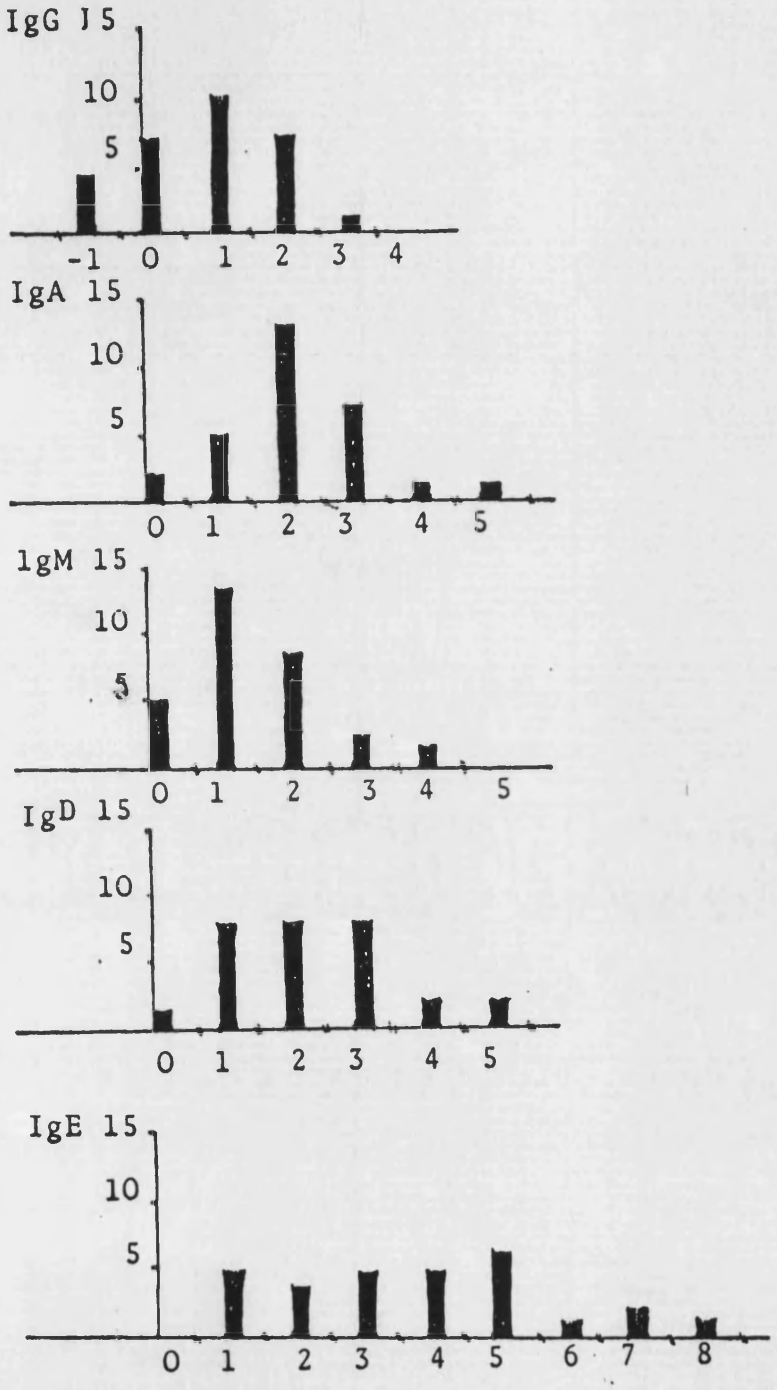
*End point of titration.
All other results measured in international units per ml.

Figure 3.3



A histogram to show the number of patients against the concentration of immunoglobulin in nasal polyp fluid (filled in) and serum (open) measured in International Units per ml.

Figure 3.4



A histogram to show the number of patients against the local production number, for the derivation of the number see text.

RAST

Standard curves may be plotted and the results of dilutions are shown below:

Standardisation Curve

HDM dilutions:	5	10	20	50	100	200	500	1000	No serum
1	3502	2566	1550	1180	635	527	293	187	115
2	3405	2235	1399	957	625	868	202	178	180
Mean	3484	2401	1475	1073	630	698	248	183	148

MGP dilutions:	5	10	20	50	100	200	500	1000	No serum
1	4980	3485	2684	1212	771	452	339	199	168
2	6476	4049	3000	1087	771	541	274	304	204
Mean	5728	3767	2842	1150	771	497	307	252	186

Background counts 55 and 13. All results were measured in mcu per minute. 50 1 of labelled anti-human IgE. 26,450 and 26,544 mcu per min.

This confirmed the binding of allergen extract to the disc paper.

All the results were duplicated and showed little variation (Table 3.4).

There was almost no raised RAST values in either polyp fluids or sera. A positive response was taken as a count of 400 or above for both HDM and MGP extract. Table 3.4 showed that in polyp fluids No. 67 had a raised RAST to HDM and that it probably was in No. 63. None of the sera was raised. Similarly, only four polyp fluids had a raised RAST to MGP: Nos. 34, 62, 63 and 67. There were no doubtful values. Of these Nos.

62 and 67 had high levels and both had a history of hay fever which was still present. Only one matched serum was raised, No. 62.

In total only four out of twenty eight patients had a raised polyp RAST to either antigen which suggested little local production of allergen specific IgE in polyp tissue to the two most common inhaled allergens. Polyp fluid values were greater than the serum levels and indicated local production of the antibody in nasal tissue. Both the patients with raised polyp RAST to HDM had positive skin tests. However, for MGP, two had positive skin tests and two had negative skin tests. The only raised RAST in serum also had a positive skin test.

Table 3.4

A table to show the RAST levels in polyp fluids and matched sera A = asthma, H = hay fever, MGP is mixed grass pollens and HDM is h dust mite. Levels above 400 m cu per min were considered positive are bracketed together.

Table 3.4:

Patient details						RAST House dust mite		RAST Mixed grass pollens	
Study No.	Skin tests A	H	HDM	MGP	Other	Polyp fluids values positive	Sera values positive	Polyp fluids values positive	Sera values positive
26	+					180	116	181	127
						156	102	231	143
29	+					133	212	182	266
						157	229	127	383
30						148	115	215	110
						96	213	126	286
31			+			157	116	402	144
						208	108	372	142
32	+					202	174	142	148
						162	157	183	199
34	+		+			217	239	537)	199
						216	276	1055)	157
36						293	140	308	150
						234	135	325	180
38						283	260	320	214
						176	213	322	112
40	+					258	118	162	129
						319	146	125	145
45						189	165	208	165
						199	85	134	124
46	+					176	129	200	177
						167	125	277	134
50			+			159	112	286	136
						290	185	180	138
58						234	141	223	127
						233	132	184	197
59				+		257	141	171	169
						176	122	223	151
60						232	143	207	150
						141	123	170	122
61						240	165	207	125
						302	150	295	125
62		+	+	+		296	303	4600)	1975)
						157	164	4700)	1855)
63			+			518)	129	445)	190
						341)	161	552)	165
64	+					129	130	201	129
						176	126	174	120
65	+	(+)	+	+		178	342	106	401
						182	326	145	273
66						163	128	138	103
						234	129	161	130
67	+	+	+	+		513)	241	1055)	260
						404)	259	1159)	271
68						356	234	303	138
						287	116	402	127
69						138	128	251	160
						173	124	145	119

RESULTS

Total IgG_4 levels (Table 3.5)

The levels of serum IgG_4 tended to be above those found in polyp fluids; the geometric mean of the serum samples was 154 μg/ml whereas it was 114 μg/ml in polyp fluids (data changed by a $\log_{10}$ transformation). Patients No 20 and 36 have high IgG_4 levels. Levels this high may be encountered in the normal population. The reason is unclear but patient 36 had dermatographia on skin tests. Twelve samples (11 patients) had a higher level of IgG_4 in the polyp fluid and this was matched by measurable levels of allergen-specific IgG_4 to HDM or MGP in eight.

Allergen-Specific (Table 3.5)

Fifteen samples (14 patients) had measurable levels of IgG_4 to MGP in both poly fluids and sera. A similar proportion of patients had measurable MGP antibody during the hay fever season or during the rest of the year (5/12 during the season approx 40%; 10/25 rest of the year approx 40%).

Measurable levels of IgG_4 antibodies to MGP were more common than HDM. There were twelve patients with raised levels to HDM; two patients had raised levels in the serà alone and in three others the corresponding values were higher in the polyp fluids.

Clinical Correlation (Table 3.6)

There was no obvious correlations between either hay fever or asthma and positive skin test results to either the total or allergen-specific levels of IgG_4.

Table 3.5: Clinical data and results of total and allergen specific levels of IgG_4 in paired sera and nasal polyp fluids from 37 patients measured in μgm/ml.

Study No.	Age	Patient details	Skin tests HDM	Skin tests MGP	Total IgG4 µg.ml Serum	Total IgG4 µg.ml Polyp	HDM Serum	HDM Polyp	MGP Serum	MGP Polyp
1	63	a s *			68	108	0	0	0.8	1.6
38	64	*			58	118	0.7	1.3	1.7	4.7
2	73	*			202	132	0	0	0	0
3	37	*			118	108	0	0	0	0
4	53	*		+	127	128	0	0	0	0
5	37	*			237	208	0	0	0	0
6	27	f *	+	+	621	200	3.8	1.3	2.3	1.3
7	81	*			140	81	0	0	0	0
8	68	a *			94	210	0	0	1.0	1.1
9	50				59	86	0	0	0	0
10	36	f			74	35	0	0	0	0
11	52				199	146	0	0	0	0
12	31	a	+	+	81	106	0	0	0.8	2.1
13	45	a h	+	+	104	90	0	0	0	0
14	51	h *		+	149	109	1.9	0.6	0	0
15	65				68	45	0	0	0	0
16	43	a *			99	102	0	0	0.7	2.1
17	43	*	+		74	78	0	0	0	0
18	34				51	69	0	0	0	0
19	66				136	131	0.5	0.6	0.8	2.8
20	37				7535	4365	4.6	0	1.6	0.3
21	34	f	+		795	227	31.5	1.5	0	0
22	32		+		156	74	0	0	0	0
23	39			+	103	142	7.3	1.6	1.6	2.3
24	62		+		127	212	0	0	3.3	6.3
25	43	h		+	101	78	0	0	0	0
26	54				128	89	0	0	0	0
27	63		+		326	188	2.7	1.0	1.5	1.0
28	62				271	147	0	0	1.1	1.5
29	53				103	66	0	0	0	0
30	51	a s	+		211	251	0.9	5.1	5.8	12.9
31	27		+		112	51	0	0	0	0
32	42				285	326	1.0	3.0	4.3	10.3
33	57		+		142	88	0	0	0	0
34	63	a	+		346	430	7.2	43.5	22.5	45.6
35	76				137	60	-	0	0	0
36	67		(D		5075	566	11.1	0	0	0)
37	54	a			172	91	0	0	0	0

a=asthmatic; h=hay fever; s=aspirin hypersensitivity; f=female
*=polypectomy performed during May to August inclusive; D=dermatographia

Table 3.6: Association between skin test results and raised levels of allergen specific IgG_4 in 36* patients

House Dust Mite

	Serum			Polyp		
	+	−	Total	+	−	Total
Skin tests:						
+	5	7	12	5	7	12
−	6	18	24	6	18	24
Total	11	25	36	11	25	36

Mixed Grass Pollens

	Serum			Polyp		
	+	−	Total	+	−	Total
Skin tests:						
+	3	4	7	3	4	7
−	11	18	29	11	18	29
Total	14	22	36	14	22	36

*one patient (36) had dermatographia and was impossible to classify.

DISCUSSION

Histamine

Histamine (β imidazolylethylamine) is formed by the dicarboxylation of L histidine which fluoresces in a similar manner to histamine and so is removed in the preparation of the sample after deproteination. Histamine is considered here as the index of mast cell degranulation although the prostaglandins and leukotrines are physiologically more active than histamine. Histamine is preformed and present in much greater quantities. It is released on challenge whereas the arachidonic acid metabolites are generated, these products are produced some 30 minutes later (Padawer 1979).

The actions of histamine are complex and it may act directly on capillary endothelium or through H_1 and H_2 receptors. The H_2 action appears confined to the gut and lungs (Black et al,1972), although there is some evidence that there are H_2 receptors in the cat's nasal mucosa. In the respiratory tract H_1 action is threefold, firstly via the smooth muscle of the bronchioles where it narrows the airway, secondly also relaxes vasculature smooth muscle and thirdly acts on capillary endothelium increasing permeability. In man it is the capillary effect which is most important. These structures are devoid of smooth muscle and the action is directly on the endothelium. The cells separate and this allows plasma proteins and electrolysis to leak through the basement membrane into the extra-cellular space (Majno et al,1969).

The blood supply of the nose is best developed over the inferior and middle turbinate with well developed venus sinusoids and capillaries (pseudo erectile tissue) and is least well developed in the sinuses; the

ethmoids are in direct contact with the nasal cavity over a large area. Since the nose is a bony box devoid of constricting smooth muscle, changes in airflow are due to changes in pooled blood in the venules, sinusoids and capillary network and then indirectly the amount of fluid present in the extracellular space.

Histamine is inactivated locally by oxidative deamination by non-specific enzymes that deaminate various aromatic and aliphatic diamines and are present in most tissues. It may also be neutralised by eosinophils which are attracted by eosinophil chemotactic factor generated by mast cell degranulation and migrate from the blood into the tissues. If histamine enters the circulation it is inactivated in the lungs and liver by methylation and the serum level remains remarkably constant.

A dynamic equilibrium exists between mast cell degranulation, local detoxification by histaminases and eosinophils and the systemic detoxification produced in the lungs and liver. If this is lost a chronic imbalance may occur leading to a prolonged oedema.

The ultrastructure of the mast cells found in nasal polyps and studied in the previous section suggests that degranulation had occurred and probably was still occurring. The findings here that there were considerable but variable amounts of free histamine in the extracellular fluid many times the serum values, suggested that this was occurring and that also the amount present had overcome local efforts to detoxify it. Partly on anatomical grounds and partly since the blood supply is less well developed than in other parts of the nose, when a chronic oedema persists, the lining of the ethmoid sinus prolapses out of its ostium and forms a polyp.

Alteration in the connective tissue ground substance has been postulated as the cause of polyposis (Smith,1971). However, more detailed analysis of the collagen types suggested no abnormality and that the changes encountered were consistent with recent inflammation (Jackson and Arihood,1971). This confirms earlier extensive histological examination of polyp tissue by Taylor where no abnormality of mucopolypsaccharides could be found and changes which Smith noted were due to non-specific staining. Taylor concluded that since polyps were so uniform in character the changes were due to oedema. If this were so then the oedema seen should be produced by vascular leakage secondary to a local tissue reaction (Taylor,1963).

Proteins

Nasal polyp oedema fluid is easy to extract and has been done so previously (Berdal,1954; Donovan et al,1970). The resulting straw-coloured fluid appeared similar to serum. Occasionally a small fibrin clot was produced in preparation to polyp fluid. Since there are few blood vessels within the polyp stroma the majority of fluid was interstitial. Results show that the total protein and albumin levels were significantly lower in polyp fluid in all cases and for both were roughly 80% of the serum value. It would appear that both large and small protein molecules leaked through a loosely adherent capillary endothelium and penetrated the basement membrane.

Electrophoresis gives protein fractions according to charge and it is possible to divide proteins into six main groups of 13 plasma proteins which comprise 95% of the serum protein mass. Each of the six sub-groups contains between two and six components. The method of

agarose gel electrophoresis allows inspection of these components and it is possible to detect any grossly abnormal concentrations of proteins. It appeared that the proteins in polyp fluid, although the levels were less, were similar in distribution in most respects to the matched serum.

Immunoglobulins

Immunoglobulins of the IgG and IgA fraction appear on electrophoresis. However this method is too insensitive to detect any but the most major deficiencies or excesses.

The respiratory epithelium of the nose produces a barrier of mucus which is continually transported backwards by cilliary action to the post nasal space where it is swallowed. The mucus contains enzymes and secretory IgA as well as much smaller quantities of other immunoglobulins. The dimeric IgA has a junctional chain and a secretory piece which makes it stable in mucus and, following reaction with antigen, forms insoluble particles. There is considerable production of IgA in the nose by local plasma cells in the submucosal layer. A rise in local production of IgG and IgM appears to be related to recent infection (Naumann,1980). IgE is found in nasal secretions in patients when symptomatic with hay fever (Huggins and Brostoff,1975).

Nasal polyps are in some respects normal respiratory mucosa and quantities of immunoglobulins should be found locally. Two studies have shown the presence of IgG, IgA, IgM and IgE in polyp fluid (Donovan et al,1970; Chandra and Abrol,1974). Both of these papers used single diffusion to measure the levels of IgG, IgA, IgM and α_2M and radioimmune assay to measure levels of IgE. These papers have two

points which may be criticised. The first is that levels of different immunoglobulins were measured by different techniques which have different sensitivities and may not be comparable and, secondly, radioimmune assays for measuring IgE may give rise to falsely high values (Nye et al,1975). Reverse passive haemagglutination overcomes these since the same techniques may be used for all measurements and the sensitivities are similar for all substances measured. There may be errors in dilution but these may be minimised with a standard technique and when done for sufficient numbers equalises out. Cross reactivity may be checked using a suitable control.

The results confirmed those of Donovan (1970) and Chandra and Abrol (1974). The level of IgA was raised over serum and suggested considerable local production. Polyp mucosa was in this respect acting like a normal respiratory mucosa: there was no evidence that a lack of IgA allowed a breakdown in the normal protection. The variable levels of IgG and IgM showed no consistent picture and suggests local production in some cases only, probably related to recent upper respiratory tract infections.

IgD has never been studied before and the exact nature of this immunoglobulin is open to speculation although it has been linked with antigen preparation and is found on the surface of macrophages and produced by plasma cells. It is interesting that there appeared to be production in all cases except one.

IgE levels are more difficult to interpret; both previous studies noticed raised IgE levels in polyp fluids. Donovan et al (1970), with twenty patients, suggested that the level was higher in 'atopic' patients but with greater numbers this study has shown that this was not

so. The raised level of IgE may be pathological but it also may reflect the normal nasal production. IgE is produced in the tonsils and adenoid tissue and to a lesser extent in the submucosa of the respiratory and alimentary tracts. Occasional plasma cells were seen in polyp tissue in the previous section. A serum level reflects overall production. Serum IgE concentrations vary widely in normal subjects, those with respiratory or nasal disease who are not atopic and those who are atopic; although the mean is significantly higher in those with atopic disease (Spitz et al,1972) Serum levels below 200 U/ml may be considered as normal (Thompson and Bird,1983). The mean serum level of the polyp patients was 100 U/ml and only five patients had levels above 200 U/ml (17%).

There was a tendency here for those with positive skin tests to have a high IgE suggesting an atopic group. There was no correlation between the serum and polyp fluid level.

Allergen specific IgE. IgE was discovered as the main immunoglobulin involved in immediate hypersensitivity in 1967 (Ishizaka and Ishizaka,1967). Soon after the RAST was developed to detect allergen specific IgE in the serum (Wide et al,1967). The place of this test in clinical practice is open to debate (Thompson and Bird,1983) but it remains a valuable research investigation. The initial enthusiasm that this test would differentiate those patients who were allergic from those who were not, has been replaced by a more balanced approach. Part of the problem has arisen from the assumption that patients were allergic rather than assuming they were not and thus proving them to be so (Stenius et al,1971; Pepys et al,1975; Bryant et al,1975; Virchow et

al,1976; Eriksson et al,1976). Studies included patients with asthma, rhinitis, urticaria and eczema either singly or more usually in combination. Part of the problem also comes from the failure to realise that asthma is a clinical condition and not a pathological or immunological diagnosis. The same too applies for nasal polyps.

When the tests are used clinically, diagnosis should initially be clinical and investigations confirm or reject this. Except in very few instances laboratory investigations aid rather than make the diagnosis. The starting point of evaluating the clinical usefulness of any test - and particularly those involved with the diagnosis of allergy - comes from clinical subgrouping. A history of symptoms on exposure would probably be allergic; a history where there is no obvious allergen would probably be non-allergic. The results of skin tests, allergen specific IgE level, total IgE levels and positive clinical challenge tests should be fitted into the clinical framework.

While studies have concentrated on RAST levels in 'allergic' subjects there are little data avalable on subjects who have no history of allergy and were either skin test positive or negative. None of the clinical studies mentioned earlier in the study had adequate control groups. This study included a small number of subjects who had no history of allergy and negative skin tests.

The assumption in this study is that although patients have nasal symptoms, they had no more allergic diseases than the normal population (hay fever, childhood asthma, penicillin hypersensitivity and eczema), then it is probable that investigations may reveal an atopic sub-group: to this extent they are normal.

Even with the limitations of previous studies it is possible to draw

some conclusions. Stenius et al (1971), studying a clinically heterogeneous group of patients, showed that there was a good correlation between high total IgE and high levels of allergen specific IgE. This study concentrated on the inhaled allergens of the dermatophagoides species (HDM) and grass pollens. These are widely accepted as the two most common causes of allergic diseases and were used in this study.

Standardisation in antigen preparation is a problem. House dust extracts contain other antigens besides those of mite although there is a great degree of overlap between the two allergen preparations (Virchow et al,1976). The greater the purity the better the response and mite is preferable to dust allergens. In this study the same allergens were used throughout both clinically and experimentally and were the standard house dust mite extract and mixed grass pollen extract (B_2) commercially available from Bencard. This necessitated the preparation of RAST rather than use commercial preparations. Results of skin tests, RASTs and challenge may then be compared directly.

The evaluation of skin tests and RAST results depend on the target organ involved. Clinically allergic patients may have a positive response on direct nasal challenge and have negative skin test (Huggins and Brostoff,1975). The evaluation of a serum allergen specific IgE level depends on the target organ size, its production of allergen specific IgE as well as the amount produced elsewhere. IgE is produced in the tonsils, adenoids and the submucosa of the respiratory and digestive tract. Since the nose is relatively small, unless the response is great, serum levels may not be elevated although local production occurs. Nasal secretions contain locally produced immunoglobulins and

may be collected. This is qualitative rather than quantitative but local production of allergen specific IgE may be demonstrated in skin test negative patients (Ibid,1975). Nasal polyp fluid may be evaluated in a quantitative fashion.

Skin tests are still widely used as a clinical test and are best performed by the prick method (Pepys,1968). Previous publications show that in symptomatic patients there is a reasonable correlation between the results of skin tests and RAST levels. The higher the RAST score the better the association with skin tests (Stenius et al,1971; Pepys et al,1975; Bryan et al,1975; Eriksson et al,1976). In either house dust mite positive skin test or raised RAST score patients, agreement is about 60-70%. There is a similar percentage for negative skin tests and a low RAST score. Agreement was better with mixed grass pollens than with house dust mite. The agreement was statistically significant for grass pollens (Eriksson et al,1976). In patients who were evidently allergic and have hay fever (Pepys et al,1975) there was a proportion of patients who have positive skin tests and a low RAST (11%). The results here showed nasal production of allergen specific IgE without a raised serum level.

Asymptomatic patients may also have positive skin tests and raised RAST scores (Huggins and Brostoff,1975). In other words, there appears to be agreement in two thirds of patients and disagreement in one third.

A previous study which measured RAST levels in polyp fluid and sera showed that more polyp RASTs were raised than sera (John and Merrett,1979) (HDM - 6 out of 46 in polyp fluid; 5 in sera; MGP - 6 out of 46 with 1 in serum). This study confirms that RAST levels are more frequently raised in polyp fluid than serum (4 cases in total, 2 HDM and

4 MGP with only 1 serum raised to MGP). This confirms that local production of allergen specific IgE occurs in the nose.

These results demonstrate that allergic reactions mediated by IgE may occur in polyp patients but, if they contribute to polyposis, they are an infrequent cause.

Short term sensitising antibodies. Some allergic reactions have been shown to be due to the IgG fraction and by the sub-class IgG_4 (Parish,1970). This immunoglobulin fraction (IgG4) differs from the IgE fraction because its activity is destroyed by heating serum to 56°C for one hour. It is eluted in the IgG fraction and is able to passively sensitise the skin of non-reacting individuals. The reactivity lasts for up to 24 hours, unlike IgE which may last for several weeks and is presumably because the mast cell receptors have low affinity (Parish,1970; Shakib and Stanworth,1980). Animal work suggests these antibodies are capable of blocking passive cutaneous anaphylaxis mediated by IgE (Stanworth and Smith,1973). Quantities of this IgG_4 are usually small compared with the other subclasses of IgG with levels of up to 0.6 mg/ml, occasionally higher values may be found. The levels are similar in both sexes (Van der Giessen et al,1975), although this has recently been disputed (French and Harrison,1984).

Initially Parish investigated the relationship between the levels of short-term sensitising antibodies to milk proteins in normal and atopic individuals (Parish,1971). All the patients had gastrointestinal symptoms and some had urticaria and asthma. Parish noted that some patients had IgG anti-milk antibodies but were tolerant of milk, which means that allergen specific can be found in asymptomatic patients.

Bryant el al investigated the role of short-term sensitising antibodies to HDM and MGP in adult asthmatics (Bryant, Burns and Lazarus,1973 and 1975). Short-term sensitising antibodies could be implicated only if the serum IgE was below 400 U/ml, if there was no raised RAST to HDM, if positive results were obtained to bronchial challenge and if the symptoms did not respond to sodium chromoglycate. Occasionally these antibodies have been implicated in children with asthma and again the therapeutic failure of sodium chromoglycate has been stressed (Gwynn et al,1978).

Both HDM and MGP levels of allergen-specific IgG_4 were found in the polyp fluid. Occasionally IgG_4 was found in amounts which would suggest local nasal production; the majority would appear to originate from the serum and to be present in lesser quantities. Twenty six patients had no evidence of allergen specific IgG_4 to HDM and twenty three no evidence for IgG_4 to MGP. Since the antibody is short term it is noteworthy that there was no difference between the summer and winter proportions who had a raised level to MGP.

These results together with the levels of allergen specific IgE for these commonest inhaled allergens would suggest little evidence for an allergic reaction.

In conclusion, the work presented in this section has demonstrated that histamine, a mast cell mediator, was present in the interstitial fluid. The nature of the fluid has been debated, however analysis of its total protein and electrophoretic mobility showed that it was essentially dilute plasma. Histamine affects the capillaries in man and allows transudation of plasma and this fits in with the constituents of

the fluid.

Some protein may be produced locally, particularly the immunoglobulins. IgA and IgE are produced locally and the presence of IgE plasma cells was found in the previous section. Evaluation of the levels of allergen specific immunoglobulins to the two commonest allergens revealed that antibodies of the IgE and IgG_4 clases could be produced locally in a few cases. It is unlikely that allergic reactions to these occur in the majority of cases.

The next section examines whether challenge to polyp tissue and peripheral blood will release histamine to these common allergens.

PART FOUR

THE RELEASE OF HISTAMINE FROM POLYP TISSUE AND PERIPHERAL BLOOD WHEN CHALLENGED WITH ANTI IgE HOUSE DUST MITE EXTRACT AND MIXED GRASS POLLEN EXTRACT AND COMPARED WITH POSITIVE SKIN TESTS

INTRODUCTION

This section evaluates the ability of polyp tissue and peripheral blood to release histamines on challenge with anti human IgE, HDM extract and MGP extract. The results with allergen challenge will be compared with skin tests results and will show whether there is any correlation between either polyp tissue response and peripheral blood response and positive skin tests. Challenge with anti IgE will show whether any allergen reaction is likely to cause mast cell degranulation. Little work has been published on in vitro allergen challenge so that there is little standardisation of either methods or results. The data that are available will be discussed in the methods and the results.

MATERIALS AND METHODS

Clinical materials: Polyps from thirty six patients which were removed under local anaethetic were coarsely minced and microfuged at 4500 rpm for 5 minutes to remove as much free histamine in the oedema as possible. Two patients were female, four had hay fever, five had aspirin sensitivity and eleven had positive skin tests, of these ten were positive to HDM and six to MGP. The largest subgroup was the fourteen patients who had asthma and these patients were compared with those who did not have chest disease.

Histamine was measured by the automated method of Evans et al (1973) as previously described (Drake-Lee and McLaughlan 1982).

Polyp tissue. After the tissue was microfuged to remove as much free histamine as possible, pieces of 100 mg to 300 mg were used for

challenge.

Sheep antihuman IgE was raised against myeloma PS and the antiserum was standardised by the Animal Research Centre at Babraham as a stock solution of 2 mg/ml, it was diluted in the same manner as Tyrodes solution. Extracts of HDM and MGP were dephenolised and dialyzed and then diluted 1 in 2, 1 in 5 and 1 in 10 in Tyrodes solution. Each concentration was used to challenge polyp tissue and peripheral blood and the maximum release which was not dose dependent was used throughout.

Each piece of polyp tissue was preincubated in 1 ml of Tyrodes solution for 5 minutes at 37°C. The Tyrodes solution was then removed and the histamine level measured. Tissue was then incubated with 1 ml of the challenge solution for 20 minutes at 37°C. The fluid was removed and the histamine level was measured. The residual tissue histamine was extracted by boiling the tissue in 1 ml of Tyrodes solution for 5 minutes and then the level of histamine was measured in the cooled solution.

Peripheral blood. Venous blood was placed in 10 ml lithium heparin bottles together with 10 ml of clotted blood for serum estimations. All samples were transferred to the laboratory within half an hour. Blood was thoroughly mixed and 250 µl were added to sample of PBS, of allergen extracts and of anti IgE. These samples were agitated and incubated for 30 minutes at 39°C. 700 ul of cold PBS was then added and the tubes were microfuged for 5 minutes at 4,500 rpm. The supernatant was evaluated for the histamine levels. Total blood histamine was determined by diluting 250 µl of whole blood 1 in 4 with PBS boiling for

5 minutes and after cooling the histamine in the supernatant was measured.

Percentage release. All results were expressed as a percentage of the total histamine available.

Polyp tissue $\frac{\text{Challenge release minus background in Tyrodes} \times 100}{\text{Total histamine in all samples}}$

Peripheral blood $\frac{\text{Challenge release minus background in PBS sample} \times 100}{\text{Total histamine in blood}}$

Positive results. Five members of the ENT department who had negative skin tests were challenged with test samples, twenty five members of the laboratory staff were also tested without skin tests. All peripheral blood samples released some histamine: in people with negative skin tests it was under 10% and in the laboratory staff under 15%. One study considered that levels of peripheral blood release of between 15% and 50% of the total blood histamine on challenge were probably a positive response and over 50% a definite response (Norman et al 1973). In this study a level of 15% and over was considered positive for both peripheral blood and polyp tissue. No previous quantative work has been performed on polyp or nasal tissue.

RESULTS

Table 4.1 shows the results of skin tests and histamine release from polyp tissue and peripheral blood following challenge. There was no dose response relationship. Although the results were grouped according to whether the patients were asthmatic, and there might at first sight be greater reactivity in the peripheral blood of asthmatics, this is related to whether the skin test was positive or not.

AND MIXED GRASS POLLEN (MGP)

⊙ Patient had positive skin test

Table 4.1

Figure 1 NASAL POLYP TISSUE

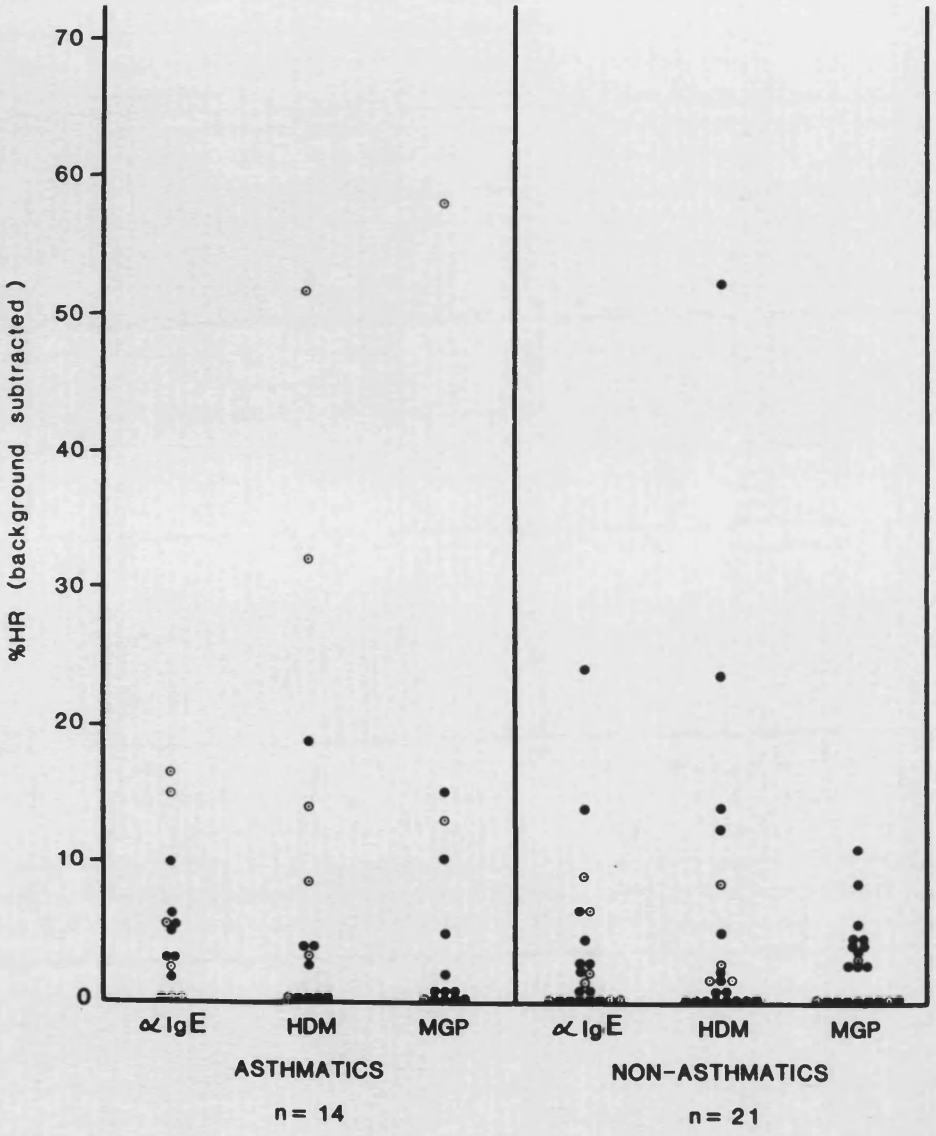
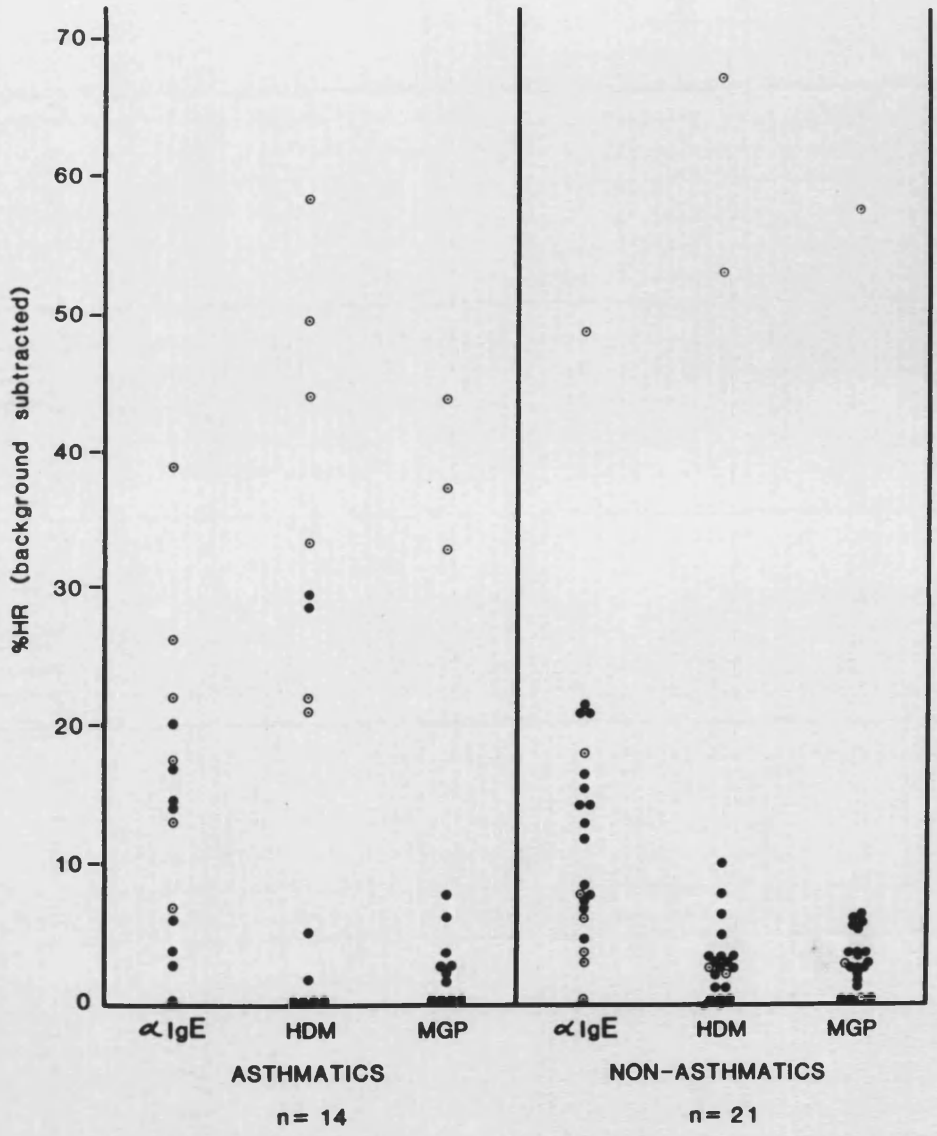


Figure 2 PERIPHERAL BLOOD



Peripheral blood. Release of histamine from peripheral blood with HDM extract was of a similar order as skin tests results. The same was true for MGP but there were fewer positive reactions. There was an association between a positive skin test and release of 15% or more of the total histamine from peripheral blood ($P<0.05$ corrected Chi square).

Anti human IgE released histamine from blood in fourteen cases but the skin test was negative in seven cases. Three of the patients who released histamine from blood did not do so with antihuman IgE.

Polyp tissue. Polyp tissue was less reactive than peripheral blood and there was no association between skin tests results and peripheral blood challenge. A positive response was obtained with anti IgE in four cases only and three released histamine with allergen challenge as well. Six of the eight patients who released histamine on allergen challenge did so without an anti IgE response. Five patients released histamine with MGP extract but of these only one of the four patients who had hay fever did so.

DISCUSSION

This section compares skin test results, peripheral blood challenge and nasal mucosal release of histamine. Mast cells are present in the connective tissue of the body and basophils are present in the circulation. Although there are differences in morphology both cell types are capable of binding IgE firmly.

Skin tests look at the reactions in the skin mast cells and the capillary basophils. Peripheral blood challenge looks at release from the circulating basophils whereas nasal tissue looks at the response

from nasal mast cells and circulating basophils. In effect three interrelated, overlaping but distinct groups of cells. Pearce (1986) believes that mast cells are both species and organ specific and yet skin tests are performed widely to test for allergic reactions.

At their best, skin tests may confirm allergy, but they do not diagnose it and indicate the tendency for allergy (atopy) within the general population. Blood challenge reflects the reactions of cells which are nearer to the site of nasal reactions than skin tests. The results here show that at the level of 15% release or more there was a good correlation between the two groups on allergen challenge but that peripheral blood samples were slightly less reactive.

Initial peripheral blood challenge studies were performed on basophils separated from the peripheral blood. These tests required at least 50 mls of blood. Total blood histamine is acceptable and a practical alternative giving a good response within 5 minutes (Norman et al 1973).

Peripheral blood challenge is not a clinical test and there is some difference in techniques and results. Norman et al using a group of grass positive patients suggested that patients with a release of total histamine above 50% were definitely and those above 15% probably positive. Radermecker (1980) who used a number of different patients and a skin test negative control group considered values above 5% were probably positive. The five skin test controls used here had levels up to 7%. Studies in infants and neonates who have yet to develop most allergic reactions showed that 10% released more than 14% of the total histamine with anti IgE (McLaughlan and Coombs 1983). This study used the values of Norman et al which would appear to be most satisfactory.

The results presented here showed that release with allergen challenge was correlated with positive skin tests results, which in the first section of the study were shown to be within the normally accepted values encountered in the British population. Release with anti IgE was found more commonly (17/36) than in infants (McLauhlan and Coombs 1983).

Virtually no work has been performed in intranasal challenge. Histamine release has been studied in children with cystic fibrosis and nasal polyps and a number of other nasal tissues including polyps in adults (Kaliner et al 1973). The study used a heterogenous group of patients and also evaluated the release of histamine from passively sensitized tissues. Kaliner et al found release from patients with hay fever and was able to induce release in the non atopic with prior incubation of tissue with serum from highly allergic patients. There were no Tyrodes controls and gave no means and ranges. Patients with hay fever released 22% $\pm$ 7% and patients who were sensitized prior to challenge released 17% $\pm$ 9%. They also did not remove the oedema fluid prior to challenge and this is important since some of the histamine has been released.

Baenkler and his colleagues (1983) reported a study which used antigen challenge to release histamine from nasal polyps. They included an anti IgE but used bacterial allergens rather than HDM and MGP. Their study may be criticised since neither was the oedema fluid removed nor were the samples preincubated to remove as much free histamine as possible. They were unable to demonstrate an IgE mediated response in the majority of patients. They expressed their results in μg/mg tissue rather than percentage release so that their results cannot be compared directly.

On the basis of peripheral blood results and the release from patients with hay fever reported by Kaliner et al a value of 15% and above was considered positive in this study.

In general polyp tissue was less reactive than either peripheral blood challenge and skin tests results. Only two cases definitely released histamine on challenge (over 50% total) and neither released histamine with anti IgE. Another six cases released more than 15% histamine available and in one this was with anti IgE alone (Table 4.1). Patients tended to release histamine both with MGP and HDM extracts.

In conclusion, nasal tissue was less reactive than peripheral blood and skin test results, it would appear allergic reactions mediated by IgE and non specific reactions to HDM and MGP pollen are unlikely to be a major cause of mast cell degranulation present in nasal polyps even though occasional responses were encountered.

CONCLUSIONS

This study has used the following hypothesis of polyp formation and tried to explore the immunocellular reactions. Mast cell reactions cause a localised oedema in the nose and sinuses. Local detoxification of vasoactive compounds depends on the tissues and their blood supply. The blood supply is very variable and is best developed within the nose and so the tissue reactions are reversed more easily than in the sinuses. The ethmoids are a special case because their complex convoluted anatomy exacerbates the condition and permits an almost irreversible herniation of the mucosa into the nose.

The cause of the tissue reactions which trigger mast cell degranulation are unknown but most authors considered that allergy is most likely. Mast cells may be degranulated by a number of stimuli and mast cell specific immunological reactions are mediated most commonly by IgE. They may also be stimulated by other non specific immunological and non immunological reactions.

Because allergy is the most widely held aetiology this study has concentrated extensively here.

The clinical section examined a population which was referred to one department and all cases which required surgery were studied. Hay fever, eczema, penicillin allergy, childhood asthma and the results of skin tests were present only as commonly expected in a normal population. There was also no obvious subgroup who had allergic disease and more severe recurrences; this was supported as far as possible in children with cystic fibrosis.

Although infection does occur in the maxillary sinuses there was

little evidence that it occurred in the majority of cases and it was probably secondary to the underlying pathology, particularly since corticosteroids are widely used in the treatment and the condition responds in half the cases.

Histological examination showed mast cells could be demonstrated by different fixation, and there were few in the epithelium. Half of the mast cells had surface IgE. The ultrastructure confirmed that mast cells were degranulated and that the process had extended onto the inferior turbinate in two thirds of the cases examined.

Electrophoresis demonstrated similar proteins as serum and that the oedema was produced by capillary leakage. Immunoglubins were present and IgA and IgE were produced in significant quantities in the polyp and this may reflect on the activity of normal respiratoy mucosa. The oedema contained vasoactive compounds and histamine levels were well above serum values.

Levels of allergen specific IgE and IgG_4 to house dust mite and mixed grass pollens (the two commonest causes of allergic rhinitis) were seldom raised. This finding together with the results of challenge of polyp tissue with allergen specific IgE suggests that allergic release of histamine is unlikely. Blanket challenge with antihuman IgE also failed to release much histamine and this result suggests that allergic reactions were unlikely to cause the mast cell degranulation found in polyps.

In conclusion, mast cell degranulation was found together with vasoactive compounds in the oedema but the trigger is unknown at present. Allergy, the widest accepted cause, seems unlikely.

REFERENCES

Anderson DH. Cystic fibrosis of the pancreas and its relation to coeliac disease: a clinical and pathological study. American Journal of the Diseases of Childhood, 1938 56:344-399

Axelson A, Grebelius N, Chidekel N. The correlation between the radiological examination and the investigation findings in maxillary sinusitis. Acta Otolaryngologica, 1970 69:302-306

Baenkler HW, W Schaubschläger, Behnsen H. Antigen induced histamine release from mucosa in nasal polyposis. Clinical Otolaryngology 1983 8:227-230

Bass RM, Potter EV, Barney PL. Immunofluorescent localization of immunoglobulins in nasal polyps. Archives of Otolaryngology, 1974 99:446-448

Berdal P. Serological examination of nasal polyp fluid. Acta Otolaryngologica, 1954 Suppl.115

Berman JM, Coleman BH. Nasal aspects of cystic fibrosis in children. Journal of Laryngology and Otology, 1977 91:133-139

Bickmore JT. Vasomotor rhinitis. An update. Laryngoscope, 1981 91:1600-1605

Billroth T. Uber den Bau der Schleimpolypen. Berlin Georg Reimer 1855 pp.36-41

Black JW, Duncan WAM, Durrant CJ, Gariellin CR, Parsons GM. Definition and antagonism of histamine H_2 receptors. Nature, 1972 236:385-390

Blumstein GI. Nasal polyps. Archives of Otolaryngology, 1966 83:266-269

Bodian M. Pathology In Fibrocystic Disease of the Pancreas. Ch 5. London:Heinemann Medical Books pp.67-146 1952

Brandtzheg P, Fjellanger I, Gjeruldsen ST. Localization of immunoglobulins in human nasal mucosa. Immunochemistry, 1967 4:57-60

Bryant D, Burns MW, Lazarus L. New type of allergic asthma due to IgG reaginic antibodies. British Medical Journal, 1973 4:589-592

Bryant D, Burns MW, Lazarus L. Identification of IgG antibody as a carrier of reaginic activity in asthmatic patients. Journal of Allergy and Clinical Immunology, 1975 56:417-428

Bryant DH, Burns MW, Lazarus L. The correlation between skin tests, bronchial provocation tests and the serum level of IgE specific for common allergens in patients with asthma. Clinical Allergy, 1975 5:145-157

Bumstead RM, El-Ackad T, Montgomery-Smith J, Brody MJ. Histamine, norepinephrine and serotinine content of nasal polyps. The Laryngoscope, 1979 89:832-843

Busuttil A, More IAR, McGeveney D. Ultrastruture of the stroma of nasal polyps. Archives of Otolaryngology, 1976 102:589-595

Caulfield JP, Lewis RA, Hein A, Austin KF. Secretions in dissociated human pulmonary mast cells. Journal of Cellular Biology, 1980 85:299-311

Cauna N, Hindover KH, Manzethi GW, Swanson EW. Fine structure of nasal polyps. Annals of Otolaryngology, 1972 81:41-58

Ceska M, Eriksson R, Varga JM. Radioimmunosorbent assay of allergens. Journal of Allergy and Clinical Immunology, 1972 49:1-9

Chandra RK, Abrol BM. Immunopathology of nasal polypi. Journal of Laryngology and Otology, 1974 88:1019-1024

Charlton R, MacKay I, Wilson R, Cole P. Double blind placebo controlled trial of beclamethasone nose drops for nasal polyps. British Medical Journal, 1985 ii:788-789

Crispin AR, Norman AP. The systematic evaluation of the chest radiographh in cystic fibrosis. Paediatric Radiology, 1974 2:101-106

Cumming G, Semple S. Bacterial content of sputum in chronic bronchitis In: Disorders of the Respiratory System. Oxford:Blackwell Scientific Publications 2nd edn pp.325-326 1980

Davies R. Diagnostic Tests. Clinical. In:Scadding JG, Cumming C, Thurlbeck WM. Scientific Foundations of Respiratory Medicine. London:William Heinemman pp.495-517 1981

Dawes J, Prichard M. Studies of the vascular arrangement of the nose. Journal of Anatomy, 1953 87:311-322

Delaney JC. Aspirin Idiosyncrasy in patients admitted for nasal polypectomy. Clinical Otolaryngology, 1976 1:27-30

Deusch LH, Drettner B. Nasal polyps treated by beclomethasone nasal aerosol. Rhinology, 1977 15:17-23

Donovan R, Johansson SGO, Bennich H, Soothill JF. Immunoglobulins in nasal polyp fluid. International Archives of Allergy and Applied Immunology, 1970 37:154-166

Doumas BT. Standards for total serum protein assays. Clinical Chemistry 1975 21:1159-1166

Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromcresol green. Clinica Chimica Acta 1971, 31:87-96

Drake-Lee AB, Pitcher-Willmott RW. The clinical and laboratory correlates of nasal polyps in cystic fibrosis. International Journal of Paediatric Otolaryngology, 1982 4:209-214

Drake-Lee AB, McLaughlan P. Clinical symptoms, free histamine and IgE in patients with nasal polyps. International Archives of Allergy and Applied Immunology, 1982 69:268-271

Dvorak AM, Galli SJ, Schulman ES, Lichtenstein L, Dvorak H. Basophil and mast cell degeneration: ultrastructural analysis of mechanisms of mediator release. Federal Proceedings 1983 42:2510-2515

Editorial. Autonomic abnormalities in asthma. Lancet 1928 (1) 1224-1225

Ehrlich P. Beitrage zur kenntnis der granulitzen bindegewebszellen und der eosinophilen leukocyten. Archives of Anatomy and Physiology (Leipzig) 1879 3:166-167

Enerback L. The gut mucosal mast cells. Monographs of Allergy. 1981 17:222-232

Ericsson NE, Ahlstedt S, Belin L. Diagnosis of reaginic allergy with house dust, animal dander and pollen allergens in adult patients. International Archives of Allergy and Applied Immunology, 1976 52:335-346

Evans DP, Lewis JA, Thompson DS. An automated fluometric assay for the rapid determination of histamine in biological fluids. Life Science, 1973 12:327-330

Fanconi G, Uehlinger E, Knauer C. Das coeliakiesyndrom bei angeborener zysticher pancreasfibromatose und bronchiectasein. Wein med Woghenschr 1936 86:753-756

Francis C. The prognosis of operations for removal of nasal polyps in cases of asthma. Practitioner 1929, 123:273-279

French MA, Harrison G. Serum IgG subclass concentrations in healthy adults: a study using monoclonal antisera. Clinical and Experimental Immunology, 1984 56:473-475

Freidmann I, Osbourne DA. Pathology of granulomas and neoplasms of the nose and paranasal simuses. Chapter 2 Miscellaneous granulomas and nasal polyposis. 1982 Edinburgh:Churchill Livingstone

Galli SJ, Dvorak AM, Dvorak HF. Mast cell activation and release. In: Progress in Allergy Vol.34 (Ed. Ishizaka K.) Chapter 1. Basophils and mast cell: morphological insights into their biology, secretory patterns and function. Basel:Karger 1944

Gibbons IR, Grimstone AV. Flagellar structure in certain flagettates. Journal of Biophysics, Biochemistry and Cytology, 1963, 7:697

Gwaltney J, Hayden F. The Nose and Infection. In: The Nose. Upper airway physiology and the atmospheric environment. (Eds Proctor D, Anderson I) C.16 pp.399-422 Amsterdam:Elsevier 1982

Gwynn CM, Morrison Smith J, Leon Leon G, Stanworth DR. Role of IgG_4 subclass in childhood allergy. The Lancet, 1978 1:910-911

Hardy W, Westbrook F. The wandering cells of the alimentary canal. Journal of Physiology, 1895 18:490-524

Harland RW, McBride NA. Allergic respiratory syndrome in general practice. Proceedings of British Students Health Association. 1974 18-23

Hiley C, Wilson H, Yates M. Identification of adrenoceptors and histamine receptors in cat nasal vasculature. Acta Otolaryngologica, 1978 85:444-448

Hopwood D. Fixation and fixatives. In: Theory and Practice of Histological Techniques. (Eds Bancroft J, Stevens A) Chapter 2 Edinburgh:Churchill Livingstone pp.20-40 1982

Huggins KG, Brostoff J. Local production of specific IgE antibodies in allergic rhinitis patients with negative skin tests. Lancet 1975 II:148-150

Hughes RG. The role of radical surgery in the treatment of recurrent nasal polyposis. Journal of Laryngology and Otology. 1973 87:117-122

Ishizaka K, Ishizaka T. Identification of gamma E antibodies as a carrier of reaginic activity. Journal of Immunology 1967, 99:1187-1198

Jackson RJ, Arihood SA. The acid mucopolysaccharides and collagen content of human nasal polyps, and peripheral nasal mucosa. Annals of Otolaryngology, 1971 80:586-592

Jeppsson JO, Laurell CB, Franzen B. Agarose gel electrophoresis. Clinical Chemistry 1979, 25:629-638

John AC, Merrett TG. The radioallergosorbent test (RAST) in nasal polyposis. Journal of Laryngology and Otology, 1979 93:889-898

Kaliner M, Wasserman SI, Austen KF. Immunologic release of chemical mediators from human nasal polyps. New England Journal of Medicine, 1973 289:277-281

Karlson G, Rundcrantz H. A randomized trial of intranasal beclomethesone diproprionate after polypectomy. Rhinology, 1982 20:144-148

Kawabori S, Umno T. Degranulation of nasal epithelial mast cells after challene of allergen. Journal of Submicroscopic Cytology, 1983 15:823-832

Kessler S, Kuhn C. Scanning electron microscopy of mast cell degranulation. Laboratory Investigation, 1975 32:71-77

Lang DA, McNeill J. Double blind controlled study of effect of topical steroids on nasal polyps. Clinical Otolaryngology 1983 8:139

Laurell CB. Antigen-antibody crossed electrophoresis. Annals of Biochemistry, 1965 10:358-361

Layton GT, Stanworth DR. The quantitation of IgG_4 antibodies to three common food allergens by ELISA with monoclonal IgG_4. Journal of Immunological Methods, 1984 73:347-356

Li C, Lam C, Yam L. Esterases in human leukocytes. Journal of Histochemistry and Cytochemistry, 1973 21:1-12

Lowe J, Bird P, Hardie D, Jefferis R, Ling N. Monoclonal antibodies (McAbs) to determinants on human gamma chains: properties of antibodies showing subclass restriction or subclass specificity. Immunology, 1982 47:329-336

Lurie MH. Cystric fibrosis of the pancreas and the nasal mucosa. Annals of Otology, Rhinology and Laryngology. 1959 68:478

Majumdar B, Bull PD. The incidence and bacteriology of maxillary sinusitis in nasal polyposis. Journal of Laryngology and Otology, 1982 96:937-941

Marsh MM, Washburn AH. Paranasal sinuses from birth to late adolescence. American Journal of Diseases of Childhood, 1940 60:841-861

Mazno G. Shea SM, Leventhal M. Endothelial contraction of cells by histamine. Journal of Cell Biology, 1969 42:647-672

McLaughlan P, Coombs RRA. Latent anaphylactic sensitivity of infants to cows milk proteins. (Histamine release from blood basophils) Clinical Allergy, 1983 13:1-9

Michels N. The Mast Cells. Handbook of Haematology. (Ed H Downey) Vol 1 pp.235-372 1938

Miller H. The structure, origin and function of mucosal mast cells: a brief review. Biologica Cellulaire, 1980 39:229-232

Moloney JR. Nasal polyps, nasal polypectomy, asthma and aspirin sensitivity. Journal of Laryngology and Otology, 1977 91:837-846

Moloney JR, Collins J. Nasal polyps and bronchial asthma. British Journal of Diseases of the Chest. 1977 71:1-6

Morris-Owen R. The natural history of asthma. In: Land DJ. Tutorials in Postgraduate Medicine: Respiratory Disease. London:Heinemman pp.225-269 1976

Mygind N. Nasal Allergy. Chapter 14, Hayfever. Oxford: Blackwell Scientific Publications 1978

Mygind N, Pedersen CB, Prytz S, Sorensen H. Treatment of nasal polyps with intranasal beclomethasone dipropionate aerosol. Clinical Allergy, 1975 5:159-164

Mygind N. The allergic inflammatory reaction. In: Essential Allergy. Oxford:Blackwell Scientific Publications pp.48-50 1986

Nakashima T, Hamashima Y. Local immune system of nasal mucosa in inflammation. IgA distribution and secretory activity. Annals of Otolaryngology, 1980 89:140-146

Naumann HH. On the defence mechanisms of the respiratory mucosa towards infection. Acta Otolaryngologica, 1980 89:165-176

Neely J, Harrison G, Jerger J, Greenberg S, Presberg H. The otolaryngologic aspects of cystic fibrosis. Trans American Academy of Ophthalmology and Otorhinolaryngology, 1972 76:313-324

Norman PS, Lichtenstein LM, Ishizaka K. Diagnostic tests in ragweed hayfever. (A comparison of direct skin tests, IgE antibody measurements and basophl histamine release). Journal of Allergy, 1973 52:210-224

Nye L, Merrett TG, Landon J, White RS. A detailed investigation of circulating IgE levels in a normal population. Clinical Allergy, 1975 5:13-24

Okuda M, Ohtsuka H, Kawabori S. Basophil leukocytes and mast cells in the nose. European Journal of Respiratory Diseases, 1983 64:(128):7-14

Padawer J. Mast cell structure: implications for normal physiology and degranulation. In: The Mast Cell (Eds Pepys J, Edwards AM) UK:Pitman Medical Chapter 1, pp.1-8 1979

Parish WE. Short term anaphylactic IgG antibodies in human sera. Lancet, 1970 11:581-592

Parish WE. Detection of reaginic and short term sensitizing anaphylactic or anaphylactoid antibodies to milk in sera of allergic and normal persons. Clinical Allergy, 1971 1:369-380

Pearce FL. On the heterogeneity of mast cells. Pharmacology, 1986 32:61-71

Pepys J. Skin tests in diagnosis. In: Clinical Aspects of Immunology Eds Gell PGH, Coombs RRA. Oxford:Blackwell Scientific Publications 1968

Pepys J, Duveen GE. Negative skin tests in allergic rhinitis and nasal polyps. International Archives of Allergy, 1951 2:147-160

Pepys J, Roth A, Carroll KB. RAST, skin and nasal tests and the history of grass pollen allergy. Clinical Allergy, 1975 5:431-442

Pfleiderer A, Drake-Lee A, Lowe D. Ultrasound of the sinuses: a worthwhile procedure? Clinical Otolaryngology, 1984 9:335-339

Pfleiderer A, Croft C, Lloyd G. Antroscopy: its place in clinical practice. A comparison of antroscopic findings with radiographic appearances of the maxillary sinus. Clinical Otolaryngology 1986, 11:455-462

Radermecker MF. Allergen-mediated histamine release from whole blood. International Archives of Allergy and Applied Immunology, 1980 63:415-423

Reynolds ES. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. Journal of Cellular Biology, 1963 17:208-212

Richardson KC, Jarrett L, Finke EH. Embedding in epoxy resin for ultra thin sectioning in electron microscopy. Stain Technology, 1960 35:313

Ritter FN. A clinical and anatomical study of various techniques of investigating the maxillary sinus. Laryngoscope, 1977 87:215-223

Samter M, Beers RF. Intolerance to aspirin. Clinical study and consideration of its pathogenesis. Annals of Internal Medicine, 1968 68:975

Samter M, Lederer FL. Nasal polyps: their relationship to allergy particularly to brochial asthma. Medical Clinics of North America, 1958 42:175-179

Schwachman H, Kukzychi IL, Mueller HL, Flake CG. Nasal polyps in patients with cystic fibrosis. Paediatrics, 1962 30:389-401

Scott ML, Merrett TG, Ishizaka K, Thornley MJ, Coombs RRA. Comparison of reverse passive anti-globulin haemagglutination with double antibody radio-immuno assay for estimation of total serum IgE. Clinical and Experimental Immunology, 1982 48:417-422

Scott M, Thornley MJ, Coombs RRA, Bradwell AR. Measurement of human serum IgE and IgA by reverse passive antiglobulin haemagglutination. International Archives of Allergy and Applied Immunology, 1981 64:222-229

Sczeklik A, Gryglewski RJ, Czerniawska-Mysik G. Relationship of inhibition of prostaglandin biosynthesis by analgesics to asthma attacks in aspirin sensitive patients. British Medical Journal, 1975 Jan 67-69

Settipane GA, Chafee FH. Nasal polyps in asthma and rhinitis. Journal of Allergy and Clinical Immunology, 1977 59:17-21

Shakib F, Stanworth D. Human IgG subclasses in health and disease. La Ricerca Clin Lab, 1980 10:561-580

Smith MP. Dysfunction of carbohydrate metabolism as the element in the set of factors resulting in the polysaccharide nose and nasal polyps. Laryngoscope, 1971 81:636-644

Solomons B. Eczema and Dermatitis. In: Lecture notes on Dermatology. Oxford:Blackwell Scientific Publications p.40 1977

Spitz E, Gelfand EW, Sheffer H. Serum IgE in clinical immunology and allergy. Journal of Allergy and Clinical Immunology, 1972 49:337-347

Spurr AR. A low viscosity epoxy resin medium for electron microscopy. Journal of Ultrastructural Research, 1962 26:31-43

Stanworth DR, Smith AK. Inhibitions of reagin-mediated PCA reactions in baboons by the human IgG_4 sub-class. Clinical Allergy, 1973 3:37-41

Stenius B, Wide L, Seymour WM, Holford-Strevens V, Pepys J. Clinical significance of specific IgE to common allergens. Clinical Allergy, 1971 I:37-55

Sternberger LA, Hardy PH, Cuculis JJ, Meyer HG. The unlabelled antibody enzyme method of immunochemistry: preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antiperoxidase) and its use in identification of spirochaetes. Journal of Histochemistry and Cytochemistry, 1970 18:315-333

Strobel S, Miller H, Ferguson A. Human intestinal mast cells: evaluation of fixation and staining techniques. Journal of Clinical Pathology, 1981 34:851-858

Sullivan TJ, Wedner HJ, Sheliz GS Yecigs LD, Parker CW. Skin testing to detect penicillin allergy. Journal of Allergy and Clinical Immunology, 1981 68:171-180

Taylor M. Histochemical studies on nasal polyps. Journal of Laryngology and Otology, 1963 77:326-341

Thompson RA, Bird AG. How necessary are specific IgE antibody tests in allergy diagnosis. Lancet, 1983 I:169-172

Trotter CM, Orr TSC. A fine structure study of some cellular components in allergic reactions. Clinical Allergy, 1973 3:411-425

Van der Giessen M, Rossouw E, Van Veen T, van Logheme, Zegers B, Sander P. Quantification of IgG subclasses in sera of normal adults and healthy children between 4 and 12 years of age. Clinical Experimental Immunology, 1975 21:501-509

Vancil ME. A historic survey of treatment for nasal polyposis. Laryngoscope, 1969 435-445

Viegas M, Gomez E, Brooks J, Gatland D, Davies RJ. Effects of pollen season on nasal mast cells. British Medical Journal, 1987 294:414

Virchow C, Roth A, Muller E. IgE antibodies to house dust mite, animal allergens and moulds in house dust hypersensitivity. Clinical Allergy, 1976 6:147-154

Von Pirquet C. Allergie. Munch Med Wochenschr, 1906 53:1457

Vourinen P, Kaupilla A, Pulkkinen EK. Comparison of results of roentgen examination and puncture irrigation of the maxillary sinuses. Journal of Laryngology and Otology, 1962 76:359-363

Watt Boulsen S, Karle A. The clinical use of radiological examination of the maxillary sinuses. Clinical Otolaryngology, 1977 2:41-43

Whiteside TL, Rabins S, Zettenberg J, Crisp L. The presence of IgE on the surface of lymphocytes in nasal polyps. Journal of Allergy and Clinical Immunology, 1975 55:186-194

Wide L, Bennich H, Johansson . Diagnosis of allergy by an in-vitro test for allergen antibodies. Lancet 1967, II:1105-1107

Wihl JA. Mast cells in the nasal mucosa. In: The Mast Cell: its role in Health and Disease. (Eds: Pepys J, Edwards AM) Bath:Pitman Press pp.758-760 1979

Wilson JA. Nasal polypi. Clinical Otolaryngology, 1976 1:4-6

Wingren U, Enerback L. Mucosal mast cells of the rat intestine: a re-evaluation of fixations and staining properties, with special reference to protein blocking and solubility of the granule glycosamene. Journal of Histochemistry, 1983 15:571-582

Woodbury D, Fingl E. Analgesic-antipyretics, anti-inflammatory agents and drugs employed in the therapy of gout. In: The Pharmalogical Basis of Therapeutics. (Eds:Goodman L, Gilman A) Chapter 17, 5th edn New York:Macmillan p.337 1975

Zuckerkandl E. Normale und pathologische anatomie der nasenhohle und ihrer pneumatischen anhange. Vol II. Wien:Wilhelm Braumuller, pp.222-246 1892